KU Leuven
Biomedical Sciences Group
Faculty of Pharmaceutical Sciences
Department of Pharmaceutical and Pharmacological Sciences

Royal Military Academy
Faculty of Social and Military Sciences
Department of Behavioral Sciences

TOWARDS AN ADEQUATE REGULATORY FRAMEWORK FOR BACTERIOPHAGE THERAPY

Gilbert VERBEKEN

Jury:

Promotors:	Prof. Dr. Isabelle Huys
	Prof. Dr. Carl Ceulemans
Co-promotors:	Prof. Dr. Rob Lavigne
	Dr. Daniel De Vos
Chair:	Prof. Dr. Arthur Van Aerschot
Jury Members:	Prof. Dr. Herman Nys
	Prof. Dr. Henri De Greve
	Prof. Dr. Sigrid Sterckx
	Prof. Dr. Martha Clokie
	Prof. Dr. João João Mendes

Dissertation presented in partial fulfillment of the requirements for the degrees of Doctor in

- Biomedical Sciences (Pharmaceutical Sciences)
- Social and Military Sciences (Behavioral Sciences)

Leuven, 28.09.2015

Table of Contents

Abbreviations

Abbreviations

AMR	Antimicrobial Resistance
ATMP	Advanced Therapy Medicinal Product
BFC	Bacteriophage Cocktail
BMP	Biological Medicinal Product
BPT	Bacteriophage Therapy
BSL	Biosafety Level
CAT	Committee for Advanced Therapies
CCPA	Court of Customs and Patent Appeals
COREC	Central Office for Research Ethics Committees
EC	Ethical Committee
ECDC	European Centre for Disease Prevention and Control
EHEC	Enterohaemorrhagic *Escherichia coli*
EIBMV	Eliava Institute of Bacteriophages, Microbiology and Virology
EJMT	Ethically Justified Medical Therapy
EMA	European Medicines Agency
EPC	European Patent Convention
EU	European Union
EUTCD	European Tissues and Cells Directive
FAMHP	Federal Agency for Medicines and Health Products
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
FOSHU	Foods for Specified Health Uses
GMP	Good Manufacturing Practices
GRAS	Generally Recognized As Safe
HE	Hospital Exemption
HIIET	Hirszfeld Institute of Immunology and Experimental Therapy
IBD	Inflammatory Bowel Disease
IP	Intellectual Property
ITF	Innovation Task Force
LabMCT	Laboratory for Molecular and Cellular Technology
LBR	Laboratory of Bacteriology Research
MDR	Multi Drug Resistant
MHRA	Medicines and Healthcare Products Regulatory Agency
MRSA	Methicillin Resistant *Staphylococcus aureus*
NATO	North Atlantic Treaty Organization
PHAGE	Phages for Human Applications Group Europe
QA	Quality Assurance
QAMH	Queen Astrid Military Hospital

QbD	Quality by Design
QC	Quality Control
QPS	Qualified Presumption of Safety
RA	Regulatory Affairs
RMA	Royal Military Academy
SME	Small and Medium-Sized Enterprises
SOP	Standard Operating Procedure
SUPERBUGS	Multi Drug Resistant Bacterial Strains
TEM	Transmission Electron Microscopy
USPTO	United States Patent and Trademark Office
WHO	World Health Organization
WMA	World Medical Association

Acknowledgement

Acknowledgement

This PhD thesis could not have been realized without the support of a number of persons and institutions.

I wish to acknowledge Prof. Dr. Isabelle Huys and Prof. Dr. Carl Ceulemans who created the frame for this co-doctorate and Dr. Jean-Paul Pirnay, Dr. Daniel De Vos and Prof. Dr. Rob Lavigne who stimulated me to step into this adventure. Isabelle and Carl also engaged themselves as my promotors, Daniel and Rob as my co-promotors.

Thanks to Dr. Geert Laire, Dr. Pierre Neirinckx, Dr. Guy Borgers, Dr. Serge Jennes and Dr. Jean-Paul Pirnay who gave me the opportunity to realize this thesis on-top of my full-time job at the Burn Wound Centre of the Queen Astrid Military Hospital, Neder-Over-Heembeek, Belgium.

I wish to acknowledge my jury members Prof. Dr. Herman Nys, Prof. Dr. Henri De Greve, Prof. Dr. Sigrid Sterckx, Prof. Dr. Martha Clokie, Prof. Dr. João João Mendes and chair Prof. Dr. Arthur Van Aerschot.

I would like to thank all informants and all interviewees whose exchange of expertise added valuable content to this work.

I wish to acknowledge the Faculty of Pharmaceutical Sciences (KU Leuven), the Department of Pharmaceutical and Pharmacological Sciences (KU Leuven), the Faculty of Social and Military Sciences (Royal Military Academy), the Department of Behavioral Sciences (Royal Military Academy), the Queen Astrid Military Hospital (Belgian Defence), the Burn Wound Centre of the Queen Astrid Military Hospital (Belgian Defence) and the Laboratory for Molecular and Cellular Biology (Burn Wound Centre, Queen Astrid Military Hospital, Belgian Defence) for giving me the opportunity to work on the bacteriophage subject, within the frame of this thesis.

Last but not least, I would like to thank my closest for their support. Especially Elsy, Emmanuel (my parents) and Igor, Katinka, Marita (my family). They were (and still are) my biggest supporters.

Acknowledgements to all persons involved in this work and who were not mentioned above. Many, many thanks to all.

Dankwoord

Dankwoord

Deze doctoraatsthesis kon nooit gerealiseerd worden zonder de steun van een aantal personen en instellingen.

Ik bedank Prof. Dr. Isabelle Huys en Prof. Dr. Carl Ceulemans die het kader voor dit doctoraat hebben gecreëerd, alsook Dr. Jean-Paul Pirnay, Dr. Daniel De Vos en Prof. Dr. Rob Lavigne die me stimuleerden om in dit avontuur te stappen. Isabelle en Carl engageerden zich tevens als mijn promotoren, Daniel en Rob als mijn co-promotoren.

Dank aan Dr. Geert Laire, Dr. Pierre Neirinckx, Dr. Guy Borgers, Dr. Serge Jennes en Dr. Jean-Paul Pirnay die me de kans gaven deze thesis te realiseren bovenop mijn voltijdse job in het Brandwondencentrum van het Militair Hospitaal Koningin Astrid, Neder-Over-Heembeek, België.

Ik bedank mijn juryleden Prof. Dr. Herman Nys, Prof. Dr. Henri De Greve, Prof. Dr. Sigrid Sterckx, Prof. Dr. Martha Clokie, Prof. Dr. João João Mendes, alsook voorzitter Prof. Dr. Arthur Van Aerschot.

Dank aan alle gesprekspartners en geïnterviewden wiens uitwisseling van expertise een waardevolle bijdrage leverde tot de inhoud van dit werk.

Dank tevens aan de Faculteit Farmaceutische Wetenschappen (KU Leuven), het Departement Farmaceutische en Farmacologische Wetenschappen (KU Leuven), de Faculteit Sociale en Militaire Wetenschappen (Koninklijke Militaire School), het Departement Gedragswetenschappen (Koninklijke Militaire School), het Militair Hospitaal Koningin Astrid (Defensie België), het Brandwondencentrum van het Militair Hospitaal Koningin Astrid (Defensie België) en het Laboratorium voor Moleculaire en Cellulaire Technologie (Brandwondencentrum, Militair Hospitaal Koningin Astrid, Defensie België) om me de kans te geven op het thema bacteriofagen te werken, binnen het kader van deze thesis.

Ten laatste, maar daarom niet minder gemeend, bedank ik mijn meest nabije familieleden voor hun steun. In het bijzonder Elsy, Emmanuel (mijn ouders) en Igor, Katinka, Marita (mijn gezin). Zij waren (en zijn nog steeds) mijn grootste supporters.

Bijzondere dank aan alle personen betrokken bij dit werk en waarvan de naam voorafgaand nog niet werd genoemd. Véél, véél dank aan allen.

1 Introduction and objectives

1 Introduction and objectives

1.1 Resistance of bacteria to antibiotics

The treatment of infectious diseases with antibiotics is becoming increasingly challenging [Levy 2004]. The rising resistance of bacteria to antibiotics is a direct result of the excessive and improper use of these drugs in conjunction with a not yet fully understanding of the role of natural antibiotics in natural bacterial communities. The effect of antibiotics is concentration dependent (hormesis) and there natural function is more of a signalling kind than that of a defensive weapon's type [Davies 2006, Linares 2006, Couce 2009]. Antimicrobial resistance (AMR) is an increasingly serious threat to global public health. The problem is so serious that it threatens the achievements of modern medicine [WHO 2014]. In Europe, 25.000 patients die annually from untreatable infections [Ackerman 2012, Verbeken 2014a]. A good example in modern medicine, amongst others, could be the huge advances in burn wound medicine resulting in increased survival rate, as a consequence of resuscitation therapies and specific surgical interventions, but where the major reason of morbidity and mortality is still due to microbial infections, especially by increasingly untreatable bacterial infections [Pirnay 2003, Church 2006]. Other examples can be found in the field of post-surgical orthopaedic surgery, as well as a variety of nosocomial infections due to multi-drug or pan-drug resistant bacterial infections. The actual estimate of deaths attributed to AMR worldwide for the year 2050 is 10 million (Figure 1) [O'Neill 2014].

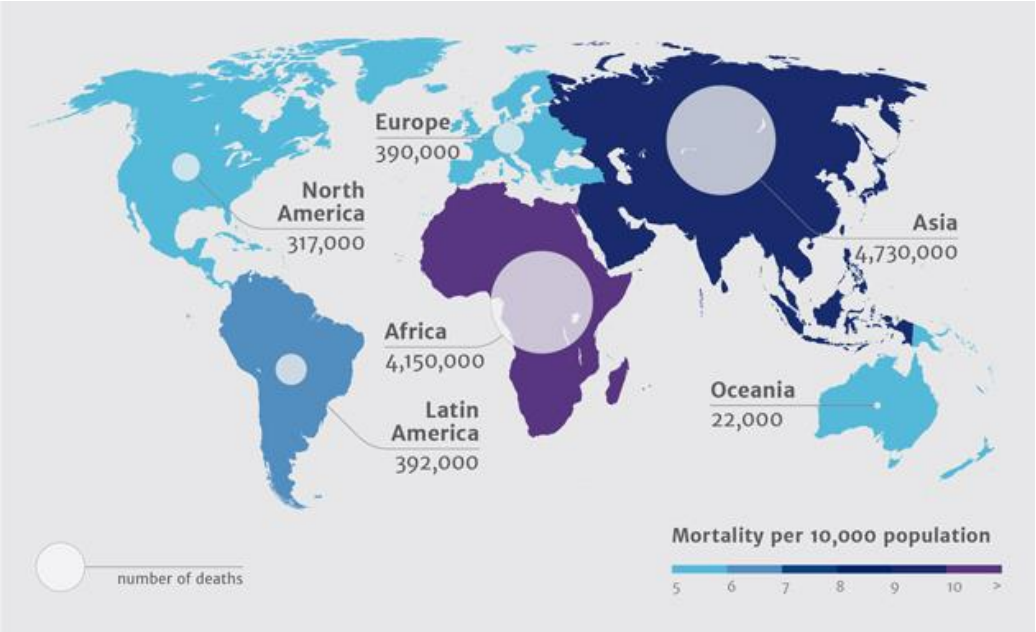


Figure 1: Deaths attributable to antimicrobial resistance every year by 2050 © Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. Review commissioned by UK Prime Minister, chaired by Jim O'Neill (December 2014) and supported by the Welcome Trust and the UK Government.

AMR also has a considerable economic impact: extra hospital costs and associated productivity losses amount to more than €1.5 billion per year in the European Union. In the USA, infections caused by multidrug resistant bacteria lead to US$20 billion in additional health-care costs and US$35 billion societal costs annually [Roberts 2009]. The continued rise of resistance would cost the world US$100 trillion by 2050 [O'Neill 2014]. Those numbers were calculated on actual available data bases containing statistical data on AMR, patients' infections rates and outcomes that where then extrapolated using two specific modelling systems in use by two multidisciplinary research teams, one from RAND Europe and another from KPMG. They looked for rising drug resistance and the consequent economic growth loss due to the labour force decline through morbidity and mortality developed[1] [O'Neill 2014]. The situation is about to deteriorate even further, as there are only a few drugs left to treat multidrug-resistant bacterial strains and the first strains that are resistant to even these last-resort antibiotics have already emerged [Wang 2006, Magoriakos 2012]. Moreover, there is a dearth of genuinely novel antibiotics in the development pipeline [Bush 2011, Huys 2013a, Verbeken 2014a].

Various proposals have been made to address the problem. These range from the more prudent use of existing antibiotics or better sanitation, to the implementation of different potential antibacterial systems based on immune system related aspects (immuno-modulators, vaccines…), to the use of new insights in bacterial lifestyles. Here an example is the development of quorum sensing inhibitors [Brackman 2011]. Other helping tools are the systematic use and integration in the diagnostic workflow of rapid new diagnostics, improving the use of different existing antibiotics and the use of the CRISPR-Cas system as a specific tool to fight bacterial infections [Horvath 2010, Rath 2015]. All these approaches should be developed in parallel and implemented in an integrated way in order to face this multi-parametric AMR problem.

However one approach seems to be promising and sustainable at the long term and implementable at the near future. That approach is "bacteriophage therapy", the use of bacteriophages to kill bacteria. Indeed, the use of bacteriophages (bacterial viruses) to kill specific bacterial pathogens without harming the majority of the commensal bacteria has received increasing attention during the past decade.

Since almost a century bacteriophages, independently discovered by Twort in UK and d'Herelle in France, are put forward and used as an antibacterial. Even before their formal discovery, the phenomenon seems to be observed by Hankin in India [Hankin 1896, Sulakvelidze 2001, Summers 2012]. The principle, set forward by d'Herelle, is using the bacterium's natural predator as weapon against a specific bacterium pathogenic for the human or animal being. Even against plant disease they could be set at work [Twort 1915, d'Herelle 1919, Thiel 2004, Sulakvelidze 2005, Bush 2011].

[1] To model incidence rates for infections today, RAND used data on the likelihood of contracting a hospital-acquired infection. They then used WHO data to calculate the average number of hospital stays in various countries and multiplied the two figures together to obtain an estimate for the number of hospital-acquired infections in each region. KPMG applied European in hospital and community infection rates to the whole world in the absence of better available data. As RAND did not include infections acquired outside of hospital and KPMG used European figures that are lower than the world average, both of these analyses are likely to systematically underestimate true infection rates.

Bacteriophage therapy could be complementary to the treatment with antibiotics or a potential alternative to this treatment [Comeau 2007, Verbeken 2007], but little has been done to capitalize on this interest and implement bacteriophage therapy in the clinic. The worldwide emergence of increasingly antibiotic resistant bacteria like the members of the so called ESKAPE group (*Esherichia coli, Staphylococcus aureus, Klebsielle pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa* and *Enterococcus spp.*), the "superbugs" and the dry-out of the antibiotic pipeline threaten modern society with a return to the pre-antibiotic era [Levy 2004, Bush 2011, Pirnay 2012, Blaser 2014].

1.2 Bacteriophages and bacteriophage therapy

1.2.1 What are bacteriophages?

Bacteriophages ("phages" in short) are the most abundant and ubiquitous biological entities on earth. Bacteriophages are the natural 'enemies' of bacteria. They are often 'spider- like' creatures (app. 40 times smaller than a bacterium) with a transparent box-shaped head. The bacteriophages focused on in this study belong to the *Caudovirales* consisting of the *Myoviridae*, the *Podoviridae* and the *Siphoviridae* (Figure 1) [Harper 2011].

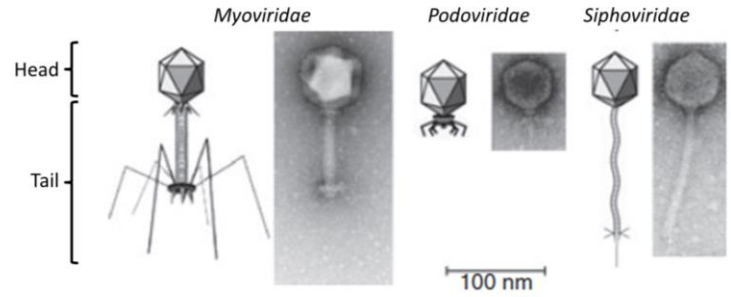


Figure 1: The tailed bacteriophages (*Caudovirales*).

Therapeutic lytic bacteriophages "take-over" the bacterium's biochemical machinery in order to produce new viral particles, called virions, after amplification of its genome and associated proteins. In practice, the bacteriophage takes up the biosynthetic machinery of the host and genetic material expression occurs resulting in directed macromolecular biosynthesis. Once the newly produced bacteriophages are assembled, specific proteins (like holins) coded for by the bacteriophage genome induce the bacteria to lyse from inside and as such liberate the virions and kill the host bacterium. The released virions could again infect a new host bacterium and reiterate the cycle. Other bacteriophages (called temperate bacteriophages) integrate (temporarily) their DNA into the bacterial chromosomal genome. The resulting lysogenic cell can replicate indefinitely, but can be induced to switch into the lytic cycle with the excision of bacteriophage DNA from the chromosome (Figure 2) [Campbell 2003, Sulakvelidze 2005]. Lysis of the host cell by bacteriophages is a complex process consisting of a cascade of events involving several structural and reguatory genes. Moreover, not all

bacteriophages replicate in a similar way. Figure 2 illustrates the standard model of the two bacteriophage cycles, the lytic and the lysogenic. This PhD thesis focusses on the therapeutic use of natural lytic bacteriophages only.

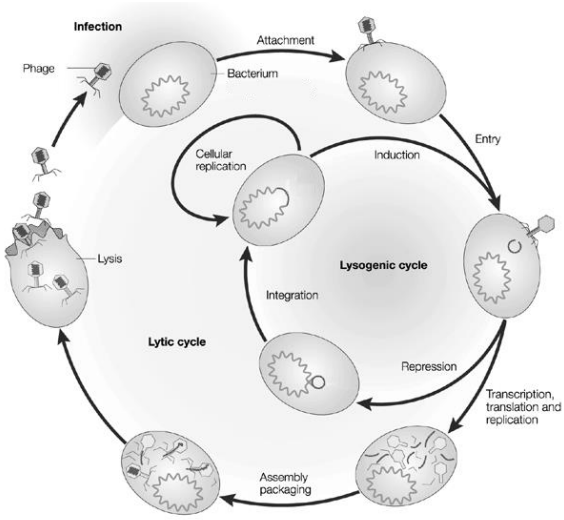


Figure 2: Life cycle of bacteriophage coliphage-λ.

Bacteriophages are bacterio-specific viruses that naturally cannot infect and replicate in a eukaryotic cell. This is due to biochemical differences such as the required polymerases that are different in eukaryotic and prokaryotic cells. To enter their bacterial host cell bacteriophages need specific outer membrane receptors beside the specific bacterial biochemical machinery for replication. Bacteriophages (derived from the Greek 'bacterio-phagein' or "bacteria eaters") keep bacterial populations growth under control. Wherever bacteria are present, there are bacteriophages which are generally present in at least a ten times higher order of magnitude than the bacteria themselves and consequently constitute the most abundant biological lifelike constituents of the biosphere of this planet [Bergh 1989, Fuhrman 1999, Hendrix 2002]. This observation shows us that we actually live in an ocean of bacteriophages and have done so since the dawn of the human species and that natural bacteriophages are in principle harmless to us, eucaryotes. A recent paper showed that bacteriophages even form a natural protection on our mucosa against bacterial invasion [Barr 2013]. Ecologically, bacteriophages are key as bacterial controllers and it is this 'natural function' of bacteriophages that bacteriophage therapy is exploiting. In combination with or as substitute for antibiotics, bacteriophages could be a therapeutic option in the eradication or control of bacterial colonisations/infections [Comeau 2007].

1.2.2 History of bacteriophage therapy

Bacteriophages were discovered independently during World War I by the English microbiologist Frederick Twort and by the French-Canadian biologist Felix d'Herelle. d'Herelle announced that nature had provided humankind with a 'living', natural weapon against bacteria. In 1919, he used

bacteriophages to treat dysentery in Paris, in what was probably the first attempt to use bacteriophages therapeutically [Pirnay 2012]. d'Herelle eventually developed a commercial laboratory in Paris that produced bacteriophage preparations against various bacterial infections, which were marketed by what later became the large French company L'Oreal [Sulakvelidze 2001]. In the 1930s, therapeutic bacteriophages were also marketed in the United States by major pharmaceutical companies including Eli Lilly, Squibb & Sons (today Bristol-Meyers Squibb) and the Swan-Meyers division of Abbott Laboratories. However, scientific controversies due to technical reasons like product purity, mismatches between bacteria and specific bacteriophages, several studies or application outcomes were discordant. Also scientifical knowledge about bacteriophage biology and bacterial biology, especially the molecular aspects, were missing. It was the time where people like Belgian Noble Prize winner Jules Bordet were claiming that the observed phenomenon was not due to a bacteriophage eliminating a bacterium, but a consequence of a kind of immune interaction. Jules Bordet challenged both the conception of bacteriophage as a virus and the effect observed as an induced lytic enzyme.

The advent of antibiotics relegated bacteriophage therapy to complete obscurity in most of the "Western World" [Summers 2012, Huys 2013a]. It was also the time that two negative reviews ordered by the Council on Pharmacy and Chemistry of the United States resulted in the cessation of commercial production of therapeutic bacteriophages [Eaton 1934, Krueger 1941, Sulakvelidze 2001, Summers 2012]. Interesting to know however is that therapeutic bacteriophage preparations could still be obtained at the Pasteur Institutes of Paris and of Lyon through the mid 1990's [Dublanchet 2009, Kutter 2010]. There continued to be reports in the literature of bacteriophage therapy applied in France until about 1979 [Vieu 1975, Vieu 1979, Kutter 2010].

Nevertheless, bacteriophage therapy was further developed and extensively used in Eastern Europe and the former Soviet Union [Sulakvelidze 2005, Summers 2012] with activities centred at the Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV) in Tbilisi, Georgia, several institutes in Russia, and the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland. Despite its long (Eastern European) history, bacteriophage therapy is not currently authorized for routine use on humans in the European Union.

1.2.3 Bacterial resistance to bacteriophages

Bacteria can evolve resistance to bacteriophages [Chanishvili 2012] through a variety of different mechanisms, including blocking bacteriophage adsorption, inhibiting the injection of bacteriophage genomes, restriction-modification systems, and abortive infection systems [Labrie 2010, Sander 2014, Rath 2015]. In *in vitro* monoculture studies, bacteriophage resistance can evolve in time frames of hours to days depending on mutation and growth rates. Whether the evolution of bacteriophage resistance *in vitro* is relevant to *in vivo* conditions where bacteria may be replicating more slowly and challenged with a greater set of environmental conditions can be questioned [Lu 2011]. Bacteriophage cocktails can delay the evolution of bacteriophage-resistance, bacteria and bacteriophages eventually reach co-existence [Kunisaki 2010, Tanji 2004]. Bacteriophage mutants

more active against the bacteriophage-resistant bacteria can be selected from the environment. Therapeutic bacteriophage banks, as they exist in Georgia and Poland, contain many different natural therapeutic bacteriophages and are regularly updated [Merabishvili 2012]. In 2015, the first modern "Western" European natural therapeutic bacteriophage bank was opened at the German Leibniz Institute - Deutsche Sammlung von Microorganism und Zellkulturen GmbH (DSMZ). Bacteriophages deposited in this special collection of the DSMZ could be of therapeutic interest if further propagated and prepared as new stocks under appropriately controlled conditions prior to their therapeutic application [DSMZ 2015]. From the environment isolated mutated bacteriophages, active against the bacteria that developed bacteriophage resistance, can be stored - for later use - in these banks. Sometimes patient-tailored bacteriophage preparations are developed [Merabishvili 2012]. This procedure is called the "Sur-Mesure" ("Tailor-Made") approach [Pirnay 2011].
The antibiotic crisis has triggered a renewed interest in the clinic, the agro-bio industry (the use of bacteriophages instead of antibiotics as growth promoters) and the food production sector [Pirnay 2012]. This, combined with new scientific insights, has pushed bacteriophages to the forefront of the search for new approaches to fighting bacterial infections.

The scope of this thesis is, seen the scientific meaningfulness and existing empiric evidence, to analyse why bacteriophage therapy is not yet a medical implementable tool in the European Union. The past years, we have realised that the main reason for its not yet routine implementation is of a regulatory kind. This thesis aims to propose a path for implementing bacteriophage therapy in the European Union, using natural bacteriophages as antibacterials in modern medicine. The bacteriophages at the centre of concern in our bacteriophage therapy concept are not bacteriophage derived products such as lysins, neither the potential use of genetically modified bacteriophages, neither products from the so called synthetic biology field.

1.2.4 Co-evolutionary concept bacterium/bacteriophage

Viruses, among which are bacteriophages, were involved in the origin of life itself and play a major role in biological evolution. Viruses played a critical role in major evolutionary transitions, such as the formation of the three domains of life (Archaea, Bacteria and Eukarya), or else, the origin of the eukaryotic nucleus [Forterre 2006, Raoult 2008]. Bacteriophage therapy, in view of our bacteriophage therapy concept, is the use of natural exclusively lytic bacterio-specific viruses as antibacterial agents. In fact, by setting up a screening system for the circulating noxious bacteria and their respective bacteriophages, it will be possible to obtain the right bacteriophage against any emerging pathogen. This way of working, taking into account the co-evolution of the couplet bacterium/bacteriophage, makes it just a fitting solution for a sustainable antibacterial bacteriophage therapy industry or a hospital-based use [De Vos 2012]. We must learn from the errors that contributed to the rise of antibiotic resistance. Any future sustainable bacteriophage therapy concept should, based on scientific grounds, fully acknowledge the potentialities of the co-evolutionary aspect of the couplet bacteriophage/bacterium in its ecological environment, *in casum* the human being. Only then the

inherent (positive) characteristics of bacteriophages as natural biological bacteria controllers can be put to use. Indeed, bacteria will inevitably become resistant to bacteriophages, but due to the continuously ongoing “arms race” between the two protagonists, specific bacteriophages able to infect the formerly resistant bacterial strains will quickly emerge. In fact bacteriophage therapy fits well in the new emerging field of Darwinian – evolutionary – medicine (in contrast to a classical mechanistic – man as a machine – view) where the insights of evolution are fully taken into account [Mayr 2004, Shanks 2007, Williams 2010, Pirnay 2011, Valenti 2012].

1.2.5 Clinical practice of bacteriophage therapy

While bacteriophage therapy can become a relevant medical option, including veterinary, agricultural, and food microbiology applications, it is for the treatment or prevention of human infections that bacteriophage therapy first caught the world's imagination and which today is the primary motivator of the field [Bruttin 2005, McVay 2007, Rhoads 2009, Harper 2010, Khawaldeh 2011, Morello 2011, Pires 2015]. Nonetheless, though the first bacteriophage therapy took place in the 1920s [d’Herelle 1917, Bruynoghe 1921], by the 1940s the field was in steep decline despite early promise. The causes were at least four-fold: (1) insufficient understanding among researchers of basic bacteriophage and bacterial biology (2) lack of purified products (3) over exuberance which led, along with ignorance, to carelessness and two camps of “believers” versus “non-believers” in bacteriophage therapy [Summers 2012] and (4) the advent of antibiotics. The latter were directly chemically well controllable products that also where easier to handle (broad spectrum activity, not necessary to know the pathogen and developable in different galenic formulations). This time point was also the starting point for the development of “modern” pharmaceutical production environments and a pharmaceutical regulatory area in which we still live. Although the classic antibiotics have really pushed medicine into a very powerful period we have to admit that today its limits are reached and that we have to adapt our system which is still fundamentally based on an end 19th century mechanical worldview, while todays (therapeutic) reality is much more dynamic. Evolution has to be integrated in medicine as a science on his own [Shanks 2007].

The decline in bacteriophage therapy was neither uniform nor complete, especially in the former Soviet Republic of Georgia, where bacteriophage therapy traditions and practice continue to this day. The advent of antibiotics and further development was more a Western industrial development that relegated bacteriophage therapy in obscurity. In the former Soviet Union however the bacteriophage therapy path was just continued. Much of the detailed knowledge we have about the practice of bacteriophage therapy comes from two places: the Republic of Georgia, especially as associated with the Eliava Institute of Bacteriophages, Microbiology and Virology, and the Hirszfeld Institute of Immunology and Experimental Therapy located in Wroclaw, Poland. The Republic of Georgia is the only place in the world where bacteriophage therapy is a component of standard medical practice, routinely used in a number of hospitals and clinics for both prophylactic and treatment purposes. Much of the bacteriophage availability, both presently and historically, has been associated with the Eliava Institute. The Hirszfeld Institute has been supplying bacteriophages to local physicians dealing

with antibiotic-resistant infections and otherwise performing bacteriophage therapy-related work for many years. In 2005, the institute established its own bacteriophage therapy clinic. The clinicians involved in bacteriophage therapy at the Hirszfeld Institute are the group most experienced with bacteriophage therapy and studying bacteriophage physiological effects found outside of the former Soviet Union [Kutter 2010]. A small number of Western physicians have been making occasional therapeutic use of bacteriophages in recent years, in Australia, Canada, France, Germany, and the USA [Abedon 2011]. A major problem has often been the obtaining of suitable bacteriophage preparations. Most of the commercially available preparations in Georgia and Russia involve very complex mixtures of bacteriophages [McCallin 2013] targeting groups of relevant bacteria. An approach that has been found clinically very effective but, it is at least assumed, would probably not be well accepted by western regulators since only based on empirism and not on actual standardized evidence based studies. Although often similar bacteriophage preparations were commercially available at the Institut Pasteur in Paris until the end of the seventies/beginning of the eighties, acquiring appropriate bacteriophage preparations to support human bacteriophage therapy is challenging today [Dublanchet 2009]. The real re-introduction of clinical bacteriophage therapy in the European Union finally will depend on the funding private companies can find today to invest in the development of bacteriophage therapy under the actual pharmaceutical regulatory framework (e.g. Biocontrol - now AmpliPhi - , Néstle, Pherecydes, Technophage, Novolytics, Intralytics…) or on the definition of a regulatory exemption under which the non-profit stakeholders can (safe and qualitatively) operate (e.g. Queen Astrid Military Hospital, Brussels, Belgium) [Soothill 2004, Bruttin 2005, Pherecydes 2013, Rose 2014, Clark 2015].

Therefore, before bacteriophage therapy can be introduced into large-scale clinical practice in the European Union, several legislative challenges (including the definition of bacteriophage specific quality, safety and efficacy guidelines) must be overcome [Loc-Carrillo 2011, Verbeken 2014a, Kutter 2015, Letkiewicz 2015]. The aim of this thesis is to propose an adapted regulatory frame for the re-introduction of bacteriophage therapy into the European Union

.

1.3 European human medicinal products legislation

For practitioners at hospitals seeking to use bacteriophages for treatment of antibiotic-resistant bacterial infections, Europe's current regulatory framework for human medicinal products hinders more than it facilitates the introduction of bacteriophage therapy in the European Union [Huys 2013b]. Although many experts consider bacteriophage therapy to be a promising complementary (or alternative) treatment to antibiotic therapy, no bacteriophage- specific regulatory framework exists to date. Today, bacteriophage therapy is only approved in some former Soviet Republics like Russia and Georgia [Chanishvili 2009]. In Poland, a recent member of the European Union, bacteriophage therapy is considered an 'Experimental Treatment' covered by the Physician Practice Act (Polish Law Gazette N° 28 of 1997) and the Declaration of Helsinki, where other therapeutic options do not exist [Górski 2009]. In France, therapeutic made-to-order bacteriophage preparations from the Institut Pasteur (Paris and Lyon) were used until the beginning of the nineties. Historical clinical data on

bacteriophage therapy (from Eastern Europe, particularly Poland, and the former Soviet Republics, particularly Georgia and Russia, as well as from today's 28 EU member states and the US) collected during the past decades are not taken into account by European regulators today [Pirnay 2010].

The current pharmaceutical economic model, implying costly and time-consuming pathways for human medicinal product development and marketing, and requiring strong intellectual property protection, is not compatible with a (possible) smooth re-introduction of traditional sustainable bacteriophage therapy into the European Union. Another major obstacle for the clinical application of bacteriophages is a false perception of viruses as 'enemies of life' [Verbeken 2007]. Bacteriophages are not straightforward inanimate and stable substances, but they are evolvable and natural biological entities. Sustainable bacteriophage therapy legislative frameworks should fully acknowledge the potential of the co-evolutionary aspect of the bacteriophage–bacterium couplet. The existing pharmaceutical regulatory framework and business models are not compatible with a dynamic and sustainable bacteriophage therapy concept. A specific European regulatory frame with realistic production and documentation requirements, which allows a timely (rapid) supply of safe, tailor-made, natural bacteriophages to patients is a must [Huys 2013a]. Fundamental changes of mentality in the medical and pharmaceutical environment are essential for a successful re-introduction of bacteriophage therapy into modern Western medicine [Pirnay 2012].

1.4 Overview of the PhD project

The clinical development of bacteriophage therapy faces major obstacles that hamper progress:

- Lack of a specific regulatory framework for bacteriophage therapy inside or outside the actual human medicinal product legal framework
- Difficulties to obtain IP-protection for bacteriophage-based products and, as a consequence, difficulties to find investors
- Absence of well-defined, safe and targeted bacteriophage preparations
- Societal misperception of viruses as 'enemies of life'

This PhD project aims at contributing to the creation of a dedicated European regulatory framework that can support the smooth re-introduction of bacteriophage therapy into the European Union. The research hypothesis is that final reflections and proposals, specifically designed in relation to bacteriophage therapy, will offer new and usable insights to all stakeholders involved.

The general objective is the assessment of the current regulatory situation in relation to the smooth implementation of bacteriophage therapy into the European Union and to formulate optimizing proposals.

The specific objectives are:

- to define the regulatory problems related to the use of therapeutic bacteriophages
- to compare the concept of bacteriophage therapy with other human medicinal products
- to discuss the bacteriophage therapy concept with stakeholders
- to define the ethical and legal (IP) issues related to bacteriophage therapy
- to formulate a proposal for a bacteriophage-specific regulatory framework for Europe

The described research is based on the following information sources:

- Literature reviews based on scientific databases (e.g. Pubmed), regulatory and legal databases (e.g. Eudralex), expert reports
- Interviews with national and international stakeholders (scientists, clinicians, pharmacists, regulators, politicians)
- Workshops (e.g. Viruses of Microbes, European Medicines Agency)
- Focus group discussions (e.g. European Commission, European Medicines Agency, Innovation Task Force, Belgian Federal Agency for Medicines and Health Products)
- Visits to bacteriophage therapy centres (e.g. Poland, Georgia)
- Master thesis projects related to bacteriophage therapy supervision

All thesis objectives are studied and discussed in detail in following chapters of this manuscript:

- Problem setting: Regulatory conundrum of bacteriophage therapy (Chapter 2)
- Development of a bacteriophage therapy concept (Chapter 3)
- Comparison of the bacteriophage therapy concept with other medicinal products and assessing the implementation of the bacteriophage therapy concept with regulatory agencies (Chapter 4)
- Defining a dedicated frame for bacteriophage therapy (Chapter 5)
- Stakeholders moral responsibility in relation to bacteriophage therapy (Chapter 6)

These 5 chapters are preceded by a general introduction (“Introduction and objectives”, Chapter 1) and followed by a general concluding discussion (“Concluding discussion”, Chapter 7).

1.5 References

Abedon S, Kuhl S, Blasdel B, Kutter E (2011) Phage treatment of human infections. Bacteriophage 1(2):66-85

Ackerman HW (2012) Who went into phage research? Bacteriophage 2:55-59

Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliaéno J, Stotland A, Wolkowicz R, Cutting AS, Doran KS et al (2013) Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc Natl Acad Sci USA 110:10771-10776

Bergh O, Borsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature 340:476-468

Blaser JB (2014) Missing microbes. Publ. Henry Holt and Company; ISBN 978-0-8050-9810-5

Brackman G, Cos P, Maes L, Nelis HJ, Coenye T (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics *in vitro* and *in vivo*. Antimicrob Agents Chemother 55(6):2655-2661

Bruttin A, Brüssow H (2005) Human volunteers receiving Escherichia coli phage T4 orally: a safe test of phage therapy. Antimicrob Agents Chemother 49(7):2874-2878

Bruynoghe R, Maisin J (1921) Essais de thérapeutique au moyen du bactériophage. Compt Rend Soc Biol 85:1120-1121

Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, Huovinen P, Jacoby GA, Kishony R, Kreiswirth BN, Kutter E, Lerner SA, Levy S, Lewis K, Lomovskaya O, Miller JH, Mobashery S, Piddock LJ, Projan S, Thomas CM, Tomasz A, Tulkens PM, Walsh TR, Watson JD, Witkowski J, Witte W, Wright G, Yeh P, Zgurskaya HI (2011) Tackling antibiotic resistance. Nat Rev Microbiol 9:894–896

Cambell A (2003) The future of bacteriophage biology. Nature Reviews I Genetics 4:471-477

Chanishvili N, Sharp R (2009) Eliava Institute of Bacteriophage,Microbiology and Virology, Tbilisi, Georgia. A literature review of the practical application of bacteriophage research. Tbilisi: Eliava Foundation

Chanishvili N (2012) A literature review of the practical application of bacteriophage research. ISBN13: 978-1621008514; ISBN-10 1621008517; 1st Edition (Book); Nova Science Publishers

Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006) Burn wound infections. Clin Microbiol Rev 19(2):403-434

Clarck J (2015) Bacteriophage therapy: history and future prospects. Future Virol 10(4):449-461

Comeau AM, Tétart F, Trojet SN, Prère MF, Krish HM (2007) Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phagegrowth. PLoS One 2(8):e799

Couce A, Blázquez J (2009) Side effects of antibiotics on genetic variability. FEMS Microb Rev 33:531-538

Davies J, Spiegelman GB, Yim G (2006) The world of subinhibitory antibiotic concentrations. Current Opinion in Microbiology 9:445-453

De Vos D, Verbeken G, Rose T, Jennes S, Pirnay JP (2012) Bacteriophages for the treatment of severe infections: a “new” option for the future? EWMA Journal 12(2):23-28

De Vos D, Verbeken G, Ceulemans C, Huys I, Pirnay JP (2014) Reintroducing phage therapy in modern medicine: the regulatory and intellectual property hurdles. In: Phage Therapy Current Research and Applications (Chapter 12); Caister Academic Press; ISBN: 978-1-908230-40-9

d'Herelle F (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. C R Acad Sci 165:373-375

d'Herelle (1919) Sur le rôle du microbe bactériophage dans la typhose aviaire. C R Acad Sci 169:932-934

DSMZ (2015) Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH / Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany http://www.dsmz.de/catalogues/catalogue-microorganisms/groups-of-organisms-and-their-applications/therapeutic-phage-bank.html

Dublanchet A (2009) Des virus pour combattre les infections: la phagothérapie. Favre: ISBN 978-2-8289-1046-4

Eaton MD, Bayne-Jones S (1934) Bacteriophage therapy. Review of the principles and results of the use of bacteriophage in the treatment of infections. JAMA 23:1769-1939

Forterre P (2006) The origin of viruses and their possible roles in major evolutionary transitions. Virus Res 117(1):5-16

Fuhrman J (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548

Górski A, Miedzybrodzki R, Borysowski J, Weber-Dabrowska B, Lobocka M, Fortuna W, Letkiewicz S, Zimecki M, Filby G (2009) Bacteriophage therapy for the treatment of infections. Curr Opin Investig Drugs 10:766–774

Hankin EH (1896) L'action bactericide des eaux de la Jumma et du Gange sur le vibrion du choléra. Ann Inst Pasteur 10:511

Harper DR, Enright MC (2011) Bacteriophages for the treatment of *Pseudomonas auruginosa* infections. Journal of Applied Microbiology 111:1-7

Hawkins C, Harper D, Burch D, Anggård E, Soothill J (2010) Topical treatment of *Pseudomonas aeruginosa* otitis of dogs with a bacteriophage mixture: a before/after clinical trial. Vet Microbiol 146(3-4):309-313

Hendrix RW. (2002) Bacteriophages: evolution of the majority. Theor Popul Biol 61(4):471-480

Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. Science 3207:167-170

Huys I, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G (2013a) Paving a regulatory pathway for phage therapy: Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. EMBO reports 14(11):951-954 DOI 10.1038/embor.2013.163

Huys I, Vaneechoutte M, Verbeken G, Debarbieux L (2013b) Key issues in phage therapy: A report of a dedicated workshop at the viruses of microbes II meeting. Res Microbiol 164(7):806-10 DOI 10.1016/j.resmic.2013.03.020

Khawaldeh A, Morales S, Dillon B, Alavidze Z, Ginn AN, Thomas L, Chapman SJ, Dublanchet A, Smithyman A, Iredell JR (2011) bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. J Med Microbiol 60:1697-1700

Krueger AP, Scribner EJ (1941) The bacteriophage: its nature and its therapeutic use. JAMA 116:2160-2167, 2269-2277; http://dx.doi.org/10.1001/jama.1941.62820190016010

Kunisaki H, Tanji Y (2010) Intercrossing of phage genomes in a phage cocktail and stable co-existence with *Escherichia coli* O157:H7 in anaerobic continuous culture. Appl Microbiol Biotechnol 85:1533-1540

Kutter E, De Vos E, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon S (2010) Phage therapy in clinical practice: treatment of human infections. Current Pharmaceutical Biotechnology 11:69-86 1389-2010/10 $55.00+.00 © 2010 Bentham Science Publishers Ltd.

Kutter E, Kuhl S, Abedon S (2015) Re-establishing a place for phage therapy in western medicine. Future Microbiol 10(5):685-688

Labrie S, Samson J, Moineau (2010) A comprehensive review of bacteriophage resistance mechanisms. Nat Rev Microbiol 8:317-327

Letkiewicz S (2015) Phage therapy of antibiotic-resistant bacterial infections as a therapeutic experiment: ethical aspects. Publ. House of the Medical University of Warsaw ISBN, 978-83-7637-322-5

Levy SB, Marshal B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:S122–S129

Linares JF, Gustafsson I, Baquero F, Martinez JL (2006) Antibiotics as intermicrobial signalling agents instead of weapons. PNAS 103(51):19484-19489

Loc-Carrillo C, Abedon S (2011) Pros and cons of phage therapy. Bacteriophage 1(2):111-114

Lu T, Koeris M (2011) The next generation of bacteriophage therapy. Curr Opin Microbiol 14(5):524-531. doi: 10.1016/j.mib.2011.07.028. Epub 2011 Aug 23

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Clinical Microbiology and Infection 18(3):268-281

Mayr E (2004) What makes biology unique? Considerations on the autonomy of a discipline. Cambridge University Press

McCallin S, Alam Sarker S, Barretto C, Sultana S, Berger B, Huq S, Krause L, Bibiloni R, Schmitt B, Reuteler G et al (2013) Safety analysis of a Russian phage cocktail: from MetaGenomic analysis to oral application in healthy human subjects. Virology 443:187-196

McVay CS, Velasquez M, Fralick JA (2007) Phage therapy of *Pseudonomas aeruginosa* infection in a mouse burn wound model. Antimicrob agents Chemother 51:1934-1938

Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M (2009) Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 4(3):e494DOI: 10.1371/journal.pone.0004944

Merabishvili M, De Vos D, Verbeken G, Kropinski A, Vandenheuvel D, Lavigne R, Wattiau P, Mast J, Ragimbeau C, Mossong J, Scheres J, Chanishvili N, Vaneechoutte M, Pirnay JP (2012) Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. PLoS ONE 7(12):e52709

Morello E, Saussereau E, Maura D, Heurre M, Touqui L, Debarbieux L (2011) Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. PLos One 6, e16963

O'Neill J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. The review on antimicrobial resistance. ISBN: 978 92 4 156474 8
http://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf

Pherecydes (2013) European project "Phagoburn" (FP7) http://www.phagoburn.eu/

Pires DP, Vilas Boas D, Sillankorva S, Azerado J (2015) Phage therapy: a step forward in the treatment of *Pseudomonas aeruginosa* infections. Journal of Virology 89(15):7449-7455

Pirnay JP, De Vos D, Cochez C., Bilocq F, Pirson J, Struelens M, Duinslaeger L, Cornelis P, Zizi M, Vanderkelen A (2003) Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J Clin Microbiol 41(3):1192-1202

Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, Zizi M, Laire G, Lavigne R, Huys I, Van den Mooter G, Buckling A, Debarbieux L, Pouillot F, Azeredo J, Kutter E, Dublanchet A, Górski A, Adamia R (2011) The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm Res 28(4):934-937 DOI 10.1007/s11095-010-0313-5

Pirnay JP, Verbeken G, Rose T, Jennes S, Zizi M, Huys I, Lavigne R, Merabishvili M, Vaneechoutte M, Buckling A, De Vos D (2012) Introducing yesterday's therapy in tomorrow's medicine. Future Virol 7(4):379-390 │10.2217/FVL.12.24©2012 Future Medicine Ltd

Pirnay JP, Blasdel BG, Bretaudeau L, Buckling A, Chanishvili N, Clark JR, Corte-Real S, Debarbieux L, Dublanchet A, De Vos D, Gabard J, Garcia M, Goderdzishvili M, Górski A, Hardcastle J, Huys I, Kutter E, Lavigne R, Merabishvili M, Olchawa E, Parikka KJ, Patey O, Pouilot F, Resch G, Rohde C, Scheres J, Skurnik M, Vaneechoutte M, Van Parys L, Verbeken G, Zizi M, Van den Eede G (2015) Quality and safety requirements for sustainable phage therapy products. Pharm Res DOI 10.1007/s11095-014-1617-7

Raoult D, Forterre P (2008) Redefining viruses: lessons from the mimivirus. Nat Rev Microbiol 6:315-319

Rath D, Amlinger L, Rath A, Ludgren M (2015) The CRISPR-Cas immune system: Biology, mechanisms and applications. Biochimie DOI:10.1016/j.biochi.2015.03.025

Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A (2009) Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care 18:237-243
Roberts RR, Hota B, Ahmad I, Scott RD 2nd, Foster SD, Abbasi F, Schabowski S, Kampe LM, Ciavarella GG, Supino M, Naples J, Cordell R, Levy SB, Weinstein RA. (2009) Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. Clin Infect Dis 49:1175–1184

Rose T, Verbeken G, De Vos D, Merabishvili M, Vaneechoutte M, Lavigne R, Jennes S, Zizi M, Pirnay JP (2014) Experimental phage therapy of burn wound infection: difficult first steps. J Burns Trauma 4(2):66-73

Sander JD, Joung JK (2014) CRISPR-Cas systems for genome editing, regulation and targeting. Nat Biotechnol 32(4):347-355 DOI:10.1038/nbt.2842

Shanks N, Pyles RA (2007) Evolution and medicine: the long reach of 'Dr. Darwin'. Philos Ethics Humanit Med 2:4

Soothill J, Hawkins C, Anggard E, Harper D (2004) Therapeutic use of bacteriophages. Lancet Infect Dis 4(9):544-545

Sulakvelidze A, Alavidze Z, Morris Jr JG (2001) Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659

Sulakvelidze A, Kutter E (2005) Bacteriophage therapy in humans. In: Kutter E, Sulakvelidze A, editors. Bacteriophages: Biology and Application. Boca Raton, Florida, CRC Press 2005:381-436
Summers WC (2012) The strange history of phage therapy. Bacteriophage 2(2):130-133 DOI:10.4161/bact.20757

Tanji Y, Shimada T, Yoichi M, Miyanaga K, Hori K, Unno H (2004) Toward rational control of Escherichia coli O157:H7 by a phage cocktail. Appl Microbiol Biotechnol 64:270-274

Thiel K (2004) Old dogma, new tricks – 21st century phage therapy. Nat Biotechnol 22:31-36

Twort FW (1915) Investigation on the nature of the ultramicroscopic viruses. Lancet 186:1241-1243

Valenti R (2012) Time, evolution and physical reductionism. EMBO rep 13:181-185

Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP (2007) European regulatory conundrum of phage therapy. Future Microbiol 2(5):485-491

Verbeken G, Pirnay JP, De Vos D, Jennes S, Zizi M, Lavigne R, Casteels M, Huys I (2012) Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine. Arch Immunol Ther Exp DOI 10.1007/s00005-012-0175-0

Verbeken G, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Huys I (2014a) Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp DOI 10.1007/s00005-014-0269-y

Verbeken G, Huys I, Pirnay JP, Jennes S, Chanishvili N, Scheres J, Górski A, De Vos D, Ceulemans C (2014b) Taking bacteriophage therapy seriously: a moral argument. Biomed Res Int 621316 DOI: 10.1155/2014/621316

Verbeken G, De Vos D, De Coninck A, Roseeuw, Kets E, Vanderkelen A, Draye JP, Rose T, Jennes S, Huys I, Ceulemans C, Pirnay JP (2015)Bacteriophage therapy, fast-forward to the past: lessons identified from the advanced therapy regulation. Burns (Submitted March 2015)

Vieu JF (1975) Les bactériophages. In: Traité de Thérapeutique; Fabre J, Ed, Flammarion, Paris, 1975, 337-340

Vieu JF, Guillermet F, Minck R, Nicolle P (1979) Données actuelles sur les applications thérapeutiques des bactériophages. Bull Acad Natl Med 163(1):61-66

Wang CY, Jerng JS, Cheng KY, Lee LN, Yu CJ, Hsueh PR, Yang PC (2006) Pandrug-resistent *Pseudomonas auruginosa* among hospitalised patients: clinical features, risk factors and outcomes. Clinical Microbiology and Infection 12(1):63-68

WHO (2014) World Health Organization: Antimicrobial resistance, global report on surveillance. http://who.int./drugresistance/documents/surveillancereport/en/

Williams PD (2010) Darwinian interventions: taming pathogens through evolutionary ecology. Trends Parasitol 26:83-92

2 Problem setting: Regulatory conundrum of bacteriophage therapy

2.1 Investigation of the regulatory and intellectual property hurdles for bacteriophage therapy (Study 1)

2.2 Development of a selection and production scheme for bacteriophages used in a clinical setting (Study 2)

2.3 The launch of a bacteriophage therapy safety trial (Study 3)

2.1 Investigation of the regulatory and intellectual property hurdles for bacteriophage therapy (Study 1)

2.1.1 Reintroducing bacteriophage therapy in modern medicine: the regulatory and intellectual property hurdles

D. De Vos, G. Verbeken, C. Ceulemans, I. Huys, J.P. Pirnay

Caister Academic Press, Chapter in a book. 2014; 289-307

International scientific journal

2.1.2 European regulatory conundrum of bacteriophage therapy

G. Verbeken, D. De Vos, M. Vaneechoutte, M. Zizi, J.P. Pirnay

Future Microbiol. 2007; 2(5):485-491

International scientific journal, peer-reviewed

2.1.1 Reintroducing bacteriophage therapy in modern medicine: the regulatory and intellectual property hurdles

D. De Vos, G. Verbeken, C. Ceulemans, I. Huys, J.P. Pirnay

Caister Academic Press, Chapter in a book. 2014; 289-307

International scientific journal

Reintroducing Phage Therapy in Modern Medicine: The Regulatory and Intellectual Property Hurdles

12

Daniel De Vos, Gilbert Verbeken, Carl Ceulemans, Isabelle Huys and Jean-Paul Pirnay

Abstract

Antibiotic resistance is a life-threatening problem worldwide and the industrial pipeline is dry. Other therapeutic options are needed and one of them is 'phage therapy'. Bacteriophages, phages in short, have proven to be effective in combating (multidrug-resistant) bacterial infections. However, legal obstacles and intellectual property rights are impeding the implementation of phage therapy in modern medicine and triggering ethical discussions. Worldwide, medicinal product regulations are directed towards standardized marketing authorization for 'classical' medicinal products. But phage are of a different nature than antibiotics on which most of our current regulation is based. Phage therapy is not covered by a specific regulatory pathway. Exceptions defined under the medicinal products legislation do not include the idea of phage therapy. Another hurdle is the Intellectual Property issue. Owning patents is essential in our current industrial economic model. But natural phages are evolving biological lifelike entities and thus difficult to cover by patents. In the future the adapted legal framework should allow the coexistence of a 'sur-mesure' pathway beside a 'prêt-à-porter' road. Taking into account the sustainability concept, all relevant safety measures and quality production controls, the 'sur-mesure' pathway should enable the use of the most fruitful and efficiency based phage therapeutic approach at regional or hospital level.

Introduction

Bacteria increasingly evolve to outsmart the available antibiotics. Yet the antibiotic pipeline is running dry while new life threatening bacterial strains are emerging continuously at an ever increasing rate (Levy and Marshal, 2004; Kumarasamy *et al.*, 2010; Brzuskiewicz *et al.*, 2011; Bush *et al.*, 2011; Cooper and Shlaes, 2011). Bacteria that are resistant to all commercially available antibiotics, so-called superbugs, are emerging worldwide. The emergence and evolution of antibiotic resistance is complex and multifactorial requiring a challenging and multidisciplinary approach if we want to control it. Indeed, this still not yet fully understood, biological phenomenon of drug resistance is a typical emergent characteristic of a dynamic, highly complex, and self-organizing system that evolves at the edge of chaos (Martinez and Baquero, 2002; Baquero *et al.*, 2003). In this setting, some laboratories and a handful of small pharmaceutical companies are (re)turning to (bacterio)phage therapy (Thiel, 2004). Phage are natural viruses that specifically infect bacteria. They are considered to be the most abundant and ubiquitous lifelike entities on Earth and co-evolve with their hosts, the bacteria (Bergh *et al.*, 1989; Fuhrman, 1999; Hendrix, 2002; Bamford, 2003). Up to 50% of bacterial mortality is thought to be due to bacteriophage predation (Wommack and Colwell, 2000). Lytic phage bind to receptors on the bacterial cell surface, inject their genetic material, use the bacterium's biochemical reproductive machinery to replicate and subsequently destroy (lyse) the bacterium, irrespective of its resistance to antibiotics, releasing the newly formed phages (virons) to seek out new hosts. It really is an evolving self amplifying antibacterial drug obviously different than a classic static chemical drug.

In 1919, d'Hérelle used phage to treat dysentery in Paris, in what was probably the first attempt to use phage therapeutically. D'Hérelle eventually developed a commercial laboratory in Paris that produced phage preparations against various bacterial infections, which were marketed by what later became the large French company L'Oreal (Sulakvelidze, 2001). In the 1930s, therapeutic phages were also marketed in the United States by major pharmaceutical companies including Eli Lilly, Squibb & Sons (today Bristol-Meyers Squibb) and the Swan-Meyers division of Abbott Laboratories. Scientific controversies and the advent of antibiotics, however, relegated phage therapy to complete obscurity in most of the Western world. Therefore, the current 'knowledge' of the therapeutic effect of phage is mainly based on theoretical grounds, basic laboratory observations, animal models and decades of empirical medical experience, accumulated mainly in Eastern Europe and the former Soviet Union with activities centered at the Eliava Institute of Bacteriophages, Microbiology, and Virology (EIBMV) in Tbilisi, Georgia, several institutes in Russia, and the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland (d'Hérelle, 1917, 1919; Sulakvelidze *et al.*, 2001; Biswas *et al.*, 2002; Wills *et al.*, 2005; Brüssow, 2005; McVay *et al.*, 2007; Chanishvili, 2009, 2012; Gorski *et al.*, 2009; Kutateladze and Adamia, 2010; Kutter *et al.*, 2010; Maura and Debarbieux, 2011). According to most supporters, phage therapy has been proven safe through the massive application of lytic bacteriophages in humans, mostly in former USSR states and Poland (Dabrowska *et al.*, 2003; Brüssow, 2005; Chanishvili, 2009), and in the Western world in animal studies (re-)initiated by UK researchers in the 1980s and reboosted by others two decades later (Smith *et al.*, 1982. Biswas *et al.*, 2002; Chibani-Chennoufi *et al.*, 2004; Wills *et al.*, 2005; Marza *et al.*, 2006; McVay *et al.*, 2007; Debarbieux *et al.*, 2010) and safety trials in healthy volunteers and patients (Brüttin and Brüssow, 2005; Rhoads *et al.*, 2009; Sarker *et al.*, 2012; McCallin *et al.*, 2013).

This chapter discusses the problems encountered when trying to reintroduce 'old school' phage therapy in our modern liberal and precautionary society.

Bacteriophage: a sustainable, evolving and self-amplifying drug

A virus is a natural biological entity, consisting of a molecular assemblage of nucleic acids (the genome) surrounded by proteins, that behaves as a genetic replicative parasite and co-evolves with its host. However, this co-evolutionary aspect, essential for sustainable phage therapy, has been practically under-estimated and consequently underutilized. Indeed, the dynamic bacteria–phage interactions (co-evolution) give rise, *in vitro* and *in vivo*, to an 'arms race', consisting of the repeated origin and fixation of new phage virulence and bacterial host defence traits (Buckling and Rainey, 2002; Faruque *et al.*, 2005a,b).

Are these natural biological 'lifelike' entities living? At first sight, viruses are 'not alive' since they are acellular and lack metabolism. On the other hand, they replicate and evolve, in a Darwinian sense, which is a typical trait of living systems. These characteristics, however, only emerge once the virion (the extra cellular viral particle or phage) has transferred its genome to an organismal cellular environment, *in casu* a bacterial cell. Research has shown that viruses, including phage, play a fundamental role in the emergence and evolution of cellular (organismal) life.

New ideas on the definition of life and the tree of life are emerging with strong debate among scientists (Ward, 2005; Cleland, 2007; Raoult and Forterre, 2008; Brüssow, 2009; Benner, 2010; Tirard *et al.*, 2010). Viruses are more and more seen as essential elements in the origin and organization of life itself (Villarreal, 2005; Villarreal and Witzany, 2010). The fundamental transition of the RNA world into the DNA world, as well as the emergence of placental organisms, was probably mediated by viruses (Mi *et al.*, 2000; Forterre, 2001). Mimivirus research brought Raoult and Forterre (2008) to conceive a new tree of life model including viruses as 'capsid encoding organisms' versus 'ribosomal encoding organisms'.

In view of these fundamental scientific developments on the nature of phage and the empirical evidence of their therapeutic usefulness, it is clear that therapeutic phage are very different from classical (chemical molecular) drugs such as antibiotics (Chanishvili, 2009; Kutateladze and

Adamia, 2010). And this challenges our actual regulatory frame which is not well adapted for phage documentation in order to setup clear and well designed standard clinical trials which are required to prove phage therapeutic effectiveness in different clinical settings as well as for the clinical optimization of this therapeutic approach (Verbeken *et al.*, 2007, 2012; Gilmore, 2012; Parracho *et al.*, 2012; Brüssow, 2012).

In fact, natural lytic phages are biological entities playing an important role in maintaining equilibrium in bacterial populations of ecological environments including man. Hence, we should not see them as a conventional chemically stable drug, but more as an interactive and evolving phage/bacterium couplet.

A phage is in fact a nucleic acid and protein based, self-amplifying and evolving medicine, exhibiting particular pharmacokinetics (Payne *et al.*, 2000; Payne and Jansen, 2003; Levin and Bull, 2004). Their action depends mainly on the susceptibility and the concentration of the targeted bacteria, the emergence of phage resistance as well as the physicochemical and immunological conditions present at the site of infection, while also the route of phage delivery, the galenic formulation and phage concentration beside the frequency and time of administration will be of importance for a successful phage therapy (Payne *et al.*, 2000; Debarbieux *et al.*, 2010; Ryan *et al.*, 2011, Morello *et al.*, 2011; Hall *et al.*, 2012).

This interactive and evolving phage/bacterium couplet, seen as a medicine, requires a new way of thinking that fits well in the emerging field of Darwinian medicine. But exactly this is less compatible with current drug development and marketing models. Indeed more than 150 years after Darwin's first publication of 'The Origin of Species' in 1859, our society as a whole, and not even the biomedical sciences as a whole, have still not well grasped and surely not yet fully integrated the idea and consequences of evolution, the cornerstone of biology, which is a basic science on its own for medicine (Darwin, 1968; Ewald, 1994; Corbellini, 2008; Williams, 2010). We agree with Shanks and Pyles that 'it is important for the public, as consumers of medical services, and for medical practitioners themselves to have a greater appreciation of the medical implications of evolutionary biology' as well as of the science of biology itself (Mayr, 2004; Shanks and Pyles, 2007). A worldview that is too deterministic and mechanistic hampers the development of the evolutionary medicine's approach as well as the consequently required adaptations of our regulatory setting and our current non-sustainable biopharmaceutical industry model. The current focus on the 'how' questions (mechanistic explanations) with immediate effects should be equilibrated with the 'why' questions (evolutionary explanations) that typically are resulting in longer term views, based on evolutionary insights (Mayr, 2004; Shanks and Pyles, 2007; Williams, 2010; Valenti, 2012).

Ethical considerations

It might be a good idea to consider some ethical issues.

Does a patient have a moral right of access to an unapproved medical treatment? Reflecting on such a question requires us to identify some of the ethical principles that seem to underlie such discussions. One of the basic principles in the realm of biomedical ethics is that of non-maleficence (Beauchamp and Childress, 2009): a minimal moral duty of any health care professional is to abstain from inflicting harm on his or her patients. There exists a general moral and legal consensus that no patient should be subjected to experimental – and therefore unapproved – medical treatments, based on this fundamental moral principle. It is necessary to conduct rigorous testing, followed by the approval by official public health agencies, in order to make sure that a proposed treatment has the beneficial health effects claimed; while at the same time it is demonstrated not to cause unacceptable health risks.

Yet is a patient's right absolute? Can they be subjected to a not (yet) approved treatment under certain conditions? If we assume that this is the case, then we need to find out under what conditions this right can justifiably be overridden. First of all, there has to be a very good reason for putting aside a patient's moral protection against being subjected to possible hazardous medical treatments. The moral weight of whatever it is we want to achieve with this therapy has to be

sufficiently important – for example, saving the life of a patient may constitute a viable ethical motive. Secondly, we need to make sure that all the moral agents involved are motivated by ethically proper intentions. If indeed a patient's life is at stake, our intention for using an unlicensed treatment has to be about improving the patient's health condition, and not about commercial, research or cost-reducing benefits. Based on the results of ongoing experimental trials, we must also have sufficient reason to believe that the treatment under investigation will indeed produce the beneficial health effects it claims to have. What is more, there must be a good prospect that the probable health benefits will outweigh the risks of subjecting the patient to the treatment.

Another criterion is that the unapproved treatment needs to be a last resort. All existing treatments must have been tried with little or no success. Finally, a decision to subject a patient to a not yet approved treatment needs to be made in respect with the patient's right to autonomy (informed consent). Notice here, however, that a patient giving his informed consent to be subjected to an unapproved treatment does not automatically lead to the waiving of his right *not* to be subjected to that therapy. A patient's moral protection against being subjected to unapproved treatment does not simply disappear because he wants it too and gives his consent as there may be ethical issues above the level of the patient that have to be considered by medical practitioners and others. Besides a patient's informed consent, all the other criteria just cited (good reason, moral intention, reasonable chance of success, proportionality, and last resort) need to be satisfied before that patient's right not to be subjected to an unapproved treatment, may justifiably be set aside.

But what if all these conditions are satisfied? Does it automatically follow from this that a patient has a *right* to access to an unapproved treatment? Well, not necessarily. If it can be shown that all these safeguards have been met, it becomes morally permissible to subject a patient to that therapy. It does not follow, however, that there exists a *duty* to provide a patient with that therapy. It might be said that the right not to be subjected to an unapproved treatment corresponds with a negative duty (duty not to harm), whereas the right to have access to an unapproved treatment corresponds with a positive duty (duty not to allow a harm to happen). Do those responsible for the development of a new treatment have such a positive duty, once it has been established that they are no longer constrained by the negative duty not to harm? Given the fact that most of those involved in developing new treatments are private companies wanting to maximize their economic profits as much as they can, it is certainly not easy to see how one could argue that such positive duty exists. A possible defence for such a duty could be based on the principle that anyone engaged in the realm of public health – and this includes the pharmaceutical companies – implicitly accepts some moral responsibilities towards society, such as making available experimental treatments in sufficiently large quantities when called for. Such a 'public responsibility'-argument is of course only one example of a possible defence, and would require a lot more development than we can provide here, but that the problem of ethical conflicts in public health research and practice exists is well known. Specifically in the area of drug resistance, as well as in the research for new antibacterial products and/or treatment approaches, ethical issues emerge at the global public health policy level, in relation with the industry, and at the individual (patient/doctor) treatment level (Aiello *et al.*, 2006; Selgelid, 2007; Leibovici *et al.*, 2012).

The Declaration of Helsinki

In 1964, the World Medical Association (WMA) elaborated a set of ethical principles for the medical community regarding human experimentation, the Declaration of Helsinki. Today, it is widely regarded as the cornerstone document of human research ethics (Bošnjak, 2001; Tyebkhan, 2003). Although it is not a legally binding instrument in international law, it draws its authority from the degree to which it has been codified in, or influenced, national or regional legislation and regulations (Human and Fluss, 2001).

In paragraph 35 of the Declaration of Helsinki ([35] of Declaration of Helsinki (2008: Sixth revision, 59th Meeting, Seoul) specifically states:

> In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

During the latter part of the 20th century, phage therapy was largely used in Poland and the former Soviet Union. One of the major centres of phage therapy is the L. Hirszfeld Institute of Immunology and Experimental Therapy, where since the 1970s specific phages have been used for the treatment of patients with suppurative bacterial infections, in whom a routine antibiotic therapy failed. The results obtained so far show that phage therapy is safe and highly effective (the majority of patients were cured) as reported by Slopek and Weber-Dabrowska (Slopek *et al*., 1987; Weber-Dabrowska *et al*., 2000).

Today, Poland is a European Union (EU) Member State in which this therapy is still possible. Phage therapy is considered an 'experimental treatment' under the Declaration of Helsinki and the national adapted Act of 5 December 1996 on the Medical Profession (Polish Law Gazette, 2011, No. 277 item 1634). On this basis the L. Hirszfeld Institute of Immunology and Experimental Therapy (an institute of the Polish Academy of Sciences) offers phage therapy to treat patients infected with drug-resistant bacteria (Miedzybrodzki *et al*., 2012).

More recently in June 2005, the Ethical Committee of the Medical Academy in Wroclaw authorized a study entitled *Experimental Phage Therapy in Antibiotic-Resistant Bacterial Infection, Including MRSA Infection*. The results of experimental phage therapy (conducted at the Phage Therapy Unit at the Hirszfeld Institute) were discussed in a recent publication (Miedzybrodzki *et al*., 2012).

Routine therapeutic application and marketing

The paragraph 35 of the Declaration of Helsinki is only applicable when therapeutic methods do not exist or have been ineffective. So, strictly speaking, the Declaration of Helsinki can only justify the application of phage in critical patients with untreatable MDR bacterial infections. Fortunately, today this is only the case for a small number of patients. As a consequence we feel that the Declaration of Helsinki is not the appropriate tool to – once and for all – evaluate the efficacy of phage therapy, which requires (according to modern medicine) prospective, double blind, placebo-controlled clinical trials, in order to eventually use phage therapy as a routine antibacterial treatment.

Medicinal product regulation

A medicinal product is defined by the European regulation as 'any substance presented for treating or preventing disease in human beings' (European Parliament, 2003). With 'substance' is meant 'any matter, irrespective of origin or intrinsic nature of that matter'. The current definition of a medicinal product is so broad that it covers a wide array of products, including foods, herbs, nutrients, micro-organisms, whole animals and even water. Consequently, leeches and fly larvae (maggots) are today indeed classified as medicinal products. Although, as previously mentioned, phage are included in the tree of life, they are definitely of a different nature than the above-mentioned 'organisms'. Nevertheless, if we strictly follow the medicinal product definition, therapeutic natural phage are undeniably medicinal products.

Based on the medicinal product regulation, they would in all probability be considered biological medicinal products, in analogy with leeches and maggots. According to some (commercial) players in the field this positioning of phage as biologicals was adopted for a transitional period of a few years. But we were not able to find any official document or report attesting this transitional phage status. At the European level, a 2008 EMEA/ECDC Technical Report 'Bacterial Challenge: Time to React' mentions phage as 'therapeutic agents', no more.

In our experience it is difficult to document phage as if they were classical medicinal products. In addition, given the specific nature of phage (i.e. natural bacterial co-evolving biological entities), we feel that it is irrational to develop and market them as if they were classical medicines (i.e. static chemical substances). In practice it is possible to produce (*ad hoc*) effective phage against current problematic bacterial infections (e.g. EHEC, NDM-1 carrying Gram-negatives, MRSA etc.) within days to weeks (Merabishvili *et al.*, 2012). If the classical medicinal product development and licensing pathways were being followed, selected organism-specific phages would only become available several years later. As a consequence, the flexibility of phage therapy would be lost (Pirnay *et al.*, 2010, 2012). Eventually, rapid updating and licensing procedures could be adopted (e.g. as in the case of influenza vaccines), but these would only reduce the postponement of use to, at best, several months. Recently Merabishvili and colleagues showed that it is possible to respond quickly on an emergent outbreak of for example the epidemic enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* (EAggEC STEC/VTEC) strain O104:H4 since the isolation, selection and characterization of a candidate therapeutic bacteriophage could be done in the timeframe of days (Merabishvili *et al.*, 2012). Therefore, a specific regulatory framework for phage therapy should be considered (Verbeken *et al.*, 2007, 2012).

In the advanced therapy medicinal product (ATMP) regulation there is a gene therapy section that allows the use of genetically modified viruses targeting eukaryotic cells. This section, however, is not applicable to natural phage, which are not genetically modified and do not target eukaryotic cells. Indeed, no phage-related nucleic acid sequence could be found in our genome, in contrast with the huge amount of retroviral remnants present in our human core genome. Up to 8–10% of the human genome consists of human endogenous retroviral sequences (HERV's) (Lander *et al.*, 2001).

Furthermore, it is impossible for a phage to interfere or multiply in an eukaryotic cell system since it requires the specific prokaryotic cell wall receptors and biochemical machinery for its attachment and replication (e.g. prokaryotic polymerases). Some phage-related polymerase gene sequences were, however, identified in human mitochondrial DNA. It is common knowledge that mitochondria originated from Rickettsia-like ancestor bacteria that started a symbiotic relationship with prototype eukaryotic cells. A similar event occurred in plants and gave rise to the chloroplast. This evolutionary process dates back from the endosymbiotic era, the time when the evolutionary split occurred between the pro- and eukaryotes. This remnant of phage DNA was likely introduced in the eukaryotic cell during the bacterial phase of the mitochondrion, the actual energy production unit of our cells. Recent work also suggests that even the eukaryotic nucleus is a viral import (Bell *et al.*, 2001). Very recently Jeremy Barr and colleagues published a paper showing a new kind of an, until now, unrecognized active mucosal protection system based on a symbiotic relationship between phage and metazoan hosts, including humans. The study showed the permanent and host-mucus dependent presence of an increased lytic phage density in the epithelial mucus layers protecting the host against invading bacteria (Barr *et al.*, 2013). All this taking into consideration shows that phage, and phage therapy, is intrinsically safe for us, human beings constituted of eukaryotic cells with whom the phage will not be able to interact.

Notwithstanding the unadapted regulatory status of phage therapy in the Western world (Verbeken *et al.*, 2007, 2012) some studies were recently conducted or are ongoing beside some sporadic therapeutic applications (Kutter *et al.*, 2010). These recent studies in conjunction with earlier empiric experiences show at least significant potentialities and basic patients safety.

As in burn centres all over the world, also the clinicians of the burn centre of the Queen Astrid Military Hospital in Brussels are increasingly confronted with multi-drug resistant (MDR) bacteria causing virtually untreatable infections (Pirnay *et al.*, 2003a). A leading Belgian Ethical Committee authorized a pilot clinical trial in which a Good Manufacturing Production (GMP)-like produced phage cocktail (Merabishvili *et al.*, 2009) was applied on burn wounds infected with MRSA and/or MDR *Pseudomonas aeruginosa*. In

the preparation phase of this pilot clinical trial researchers of the Brussels burn wound centre were confronted with the false perception of viruses as 'enemies of life', an observation that was previously expressed by Villarreal (2005). This 'fear', for example, resulted in a ten times too high study insurance fee and an unwarranted request to notify the National Bio-safety Council, which normally is only entitled to rule on the release of genetically modified organisms and pathogenic micro-organisms in the environment. In addition, this cocktail was also used to successfully treat a critical patient, amongst others, suffering since months from a pelvic osteomyelitis infection accompanied with frequent septic episodes, after a car crash and subsequent surgical intervention, with MRSA and MDR *P. aeruginosa* under the umbrella of the Declaration of Helsinki.

In the UK a small phage therapy company conducted a phase I/II clinical trial in chronic otitis approved, on a national level, by the UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC). A preliminary report of efficacy was published (Wright *et al*., 2009).

In France therapeutic made-to-order phage preparations from the Institut Pasteur of Paris and Lyon were available for medical use till the beginning of the nineties (Dublanchet, 2009; Kutter *et al.,* 2010). Although phage therapy was not retained in the current French Medicinal Product Regulation, a French MD, Alain Dublanchet, still applies phage therapy to treat patients infected with MDR bacterial infections. He obtains his phage products from pharmacies in Russia and/or Georgia where they are legally and commercially available (Personal communication of Dublanchet).

In the United States a Food and Drug Administration (FDA)-approved phase I clinical trial was performed (Rhoads *et al.,* 2009). A recent publication from an international group describes in a case report the application of therapeutic bacteriophages for refractory *P. aeruginosa* urinary tract infection in a 67-year-old woman that underwent extensive intra-abdominal resections and irradiation for adenocarcinoma. This treatment was approved by the Western Sydney Human Research Ethics Committee on a compassionate use basis and after patient's informed consent was obtained (Khawaldeh *et al*., 2011).

Other potential paths

Several alternative application paths for phage can be taken into consideration.

Magistral or extemporaneous preparations

At first sight, phage preparations could be produced and dispensed as Magistral or Extemporaneous preparations by a (hospital) pharmacist. The dispenser (pharmacist) stands between the prescriber and the patient and only a very intimate acquaintance with the characters and doses of medicines will enable him to successfully perform his duty to each. The successful performance of this medication has to be preceded by knowledge of the physical and chemical characters of its (active) components and it is debatable whether this is currently the case for phage. Furthermore, in most countries the raw materials of Magistral or Extemporaneous preparations need to be licensed in/or accompanied by an adequate certificate of analysis. Also the traceability has to be guaranteed in a way which conforms to current regulations.

Compassionate use

'Compassionate use' is a method of providing experimental therapeutics (investigational drugs) prior to final approval for use in humans. This procedure is used with seriously ill individuals who have no other treatment options. This rule only applies to non-registered drugs that are already in a clinical-study phase and have proven potential. Phage preparations that are currently tested in approved clinical trials and have shown efficacy could thus theoretically, and under certain conditions, be used to treat patients with life threatening MDR bacterial infections. Recently, an official question regarding the compassionate use, eventually under or in combination with the French 'statut d'Authorisation Temporaire d'Utilisation' (ATU), of therapeutic bacteriophages against life threatening resistant bacterial infections in patients with functional or vital bad prognosis was asked by two French MDs, Larché and Lenoir

from the non-profit organization PhagEspoirs to the French competent authorities. The answer is still pending. In Australia however phage was recently successfully applied under a compassionate use basis (Khawaldeh *et al.*, 2011).

GRAS and QPS

Antibiotics and other antibacterial (preservation) products play an important role in the food and agro-biological industry. More than half of the antibiotic consumption worldwide is for non-human medical use, often as animal growth promoter/enhancer. The routine and widespread (over) use of antibiotics in the agro-biological industry, which are also used in human medicine, is no longer generally accepted since it was shown to accelerate the development of antibiotic-resistant strains of bacteria. Several studies showed the presence of antibiotic-resistant strains and molecular antibiotic gene cassettes in the inanimate environment (Kümmerer and Henninger, 2003; Pirnay *et al.*, 2005; Allen *et al.*, 2010). Although recent surveys and specific studies show the widespread presence of antibiotic resistance genes in the environment, even in remote pristine environments, we do not agree to quickly change the paradigm that 'the overuse and misuse of antibiotics (OMUAB) is at the origin of the emergence of the increased antibiotic resistant bacteria' (Selgelid, 2007; Bertoloni *et al.*, 2009; Allen *et al.*, 2010; D'Costa *et al.*, 2011; Rolain *et al.*, 2012). The effect of OMUAB might indeed not be specifically at the origin of the *resistance genes* as such, but it is apparently clearly related to their *mobilization* from the so-called resistome (D'Costa *et al.*, 2006). This phenomenon is currently enhanced as a consequence of the worldwide massive use of antibiotics especially in the agrobio industry. We actually have to realize that in fact our knowledge of the natural role of antibiotics, as secondary metabolites, is incomplete or too low. They can have a signalling function(s), similar to cytokines in eukaryotes, in their natural ecological setting. Clearly, a better use of antibiotics requires more integrated and fundamental research. Research results through metagenomic studies will surely help and bring new useful insights (Schmieder and Edwards, 2012). Realizing this, it is not surprising, and even a good initiative of the food and agro-biological industry, to also try to exploit phage–host interactions for biotechnological applications (Shapiro and Kushmaro, 2011; Mahony *et al.*, 2011) in order to avoid the use of antibiotics or at least to minimize it or use it more appropriately.

Some companies decided to penetrate the food market first in order to accustom the public and the regulatory authorities to bacteriophages, which should facilitate future clinical trials whilst already generating revenues. In the USA, the Food and Drug Administration (FDA) approved the use of the first phage preparation for food safety applications in April 2006. Since then, three other preparations have been approved. FDA classified two of these preparations under the Generally Recognized As Safe (GRAS) product regulation, one was cleared as a food additive, and another one as a food contact substance (Burdock and Carabin, 2004; FDA, 2006; Sulakvelidze, 2013).

In 2009, the European Food Safety Agency (EFSA) issued a product regulation similar to GRAS, the Qualified Presumption of Safety (QPS) regulation. In addition, the EFSA maintains a list of QPS micro-organisms that can be intentionally added to food or feed. Phage are not included on the list, but similar to the GRAS regulation, they can be authorized as antimicrobial agents in the industrial food production after assessment on a case-by-case basis. The review process pays specific attention to the characterization of the phage at the genomic and proteomic level, which should ascertain that the phage are exclusively lytic and are not carrying potential toxins and/or virulence factors that are potentially transmissible in the natural ecological environment. As such two different cocktails of phages active against *L. monocytogenes* were approved and are commercially in use and available, Intralytix's ListShield and Micreos'Listex.

Phage probiotics?

Phage are extremely abundant and ubiquitous. They are present in environments as diverse as seawater, drinking water, activated sludge, food and cosmetics, and inhabit our bodies, especially the digestive tract, outnumbering our own bodies eukaryotic cell number. In the food industry phage play an important role not only in 'starter culture

mixes' but also as 'in-process agents of concern', especially in all kind of fermentation products where they can have huge negative impact on bacterial fermentation based production processes (Shapiro and Kushmaro, 2011). Unknowingly we constantly consume phage with our drinking water and food (e.g. yoghurt, cheese, salami).

An equilibrated food intake is the basis for health. Yoghurts containing probiotics such as bifidobacteria, also available on the European market, are claimed to have a positive influence on our health through the restoration of the intestinal flora. This means, in fact, that there is a commercial claim that these yoghurts generate positive therapeutic effects. However, at the regulatory level, the therapeutic effects for this type of product are not perceived, since otherwise such yoghurts would be considered medicinal products according to European regulation. Fermented-milk drinks containing living *Lactobacillus* spp. such as *Lactobacillus casei shirota* or *Lactobacillus casei immunitas*, but also their respective phage, are also present on the European market.

According to the Japanese Foods for Specified Health Use (FOSHU) regulation, the following therapeutic effects of probiotics can be claimed: 'regulation of the gastrointestinal condition', 'reduction of harmful bacteria' and 'suitable for therapeutic use against acute diarrhoea' (Sanders and Huis in't Veld, 1999; Berman *et al.*, 2006). These products did not receive the medicinal product status, either.

In The Netherlands, some milk drinks are claimed to 'improve bowel habits' in subjects who are susceptible to constipation and to 'support a well-balanced gut microbiome' through an increase in the number of *Lactobacilli*. These claims were validated by a committee of independent scientists on specific request of The Netherlands Nutrition Centre and a major milk-drink manufacturer. Despite these valorized claims, these products were not classified as a medicinal product, either (Agarwal *et al.*, 2002).

Apparently, for food products, therapeutic claims did not have to be backed up by clinical (safety) trials or, in fact, by any study whatsoever. And what to think about other, more 'classical' nutritional supplements such as various vitamins or minerals like calcium, iron or magnesium amongst others, who in fact are considered as medicinal products. This shows that although a medicinal product was defined there are several exceptions that enable to use the 'same substance' as medicinal product or non-medicinal product. However, questions have increasingly been raised regarding the status of these 'grey-zone' products. New health claim regulations in Europe, and a tightening regulatory environment in the US make it harder to make therapeutic claims. Several claims are unfavourably assessed because they were not supported by any relevant studies in humans. Today, such studies are central to the establishment of a cause and effect relationship between the food and/or substance concerned and the beneficial health effect claimed. But even with some good scientific evidence 'probiotics as therapeutics' are actually facing approval problems as described by Reid (2011).

Phage could eventually be considered as food additives with therapeutic claims based for example on the restoration of the commensal flora of the gastrointestinal tract, by eliminating pathogens which could promote the growth of commensals, but the health claim regulation and licensing pathways for food-additives are, again, not compatible with the flexibility and sustainability we pursue for phage therapy.

Intellectual property rights

As mentioned earlier the rise of multi-resistant bacterial infections shows the current limits of common antibiotics. In the bio-medical community, there is currently a renewed interest in phage therapy, but (bio)pharmaceutical companies seem generally not interested to push it forward. The main reason for this is that, apart from the lack of an appropriate regulatory framework, it is hard to get patents for phage and their therapeutic applications, hence no way to secure a return on investment costs (Thiel, 2004). The fact that intellectual property (IP) rights or patents give problems in the biomedical field is increasingly recognized (Aiello, 2006; Selgelid, 2007, Taubman, 2008; Gold *et al.*, 2009; Kapczynski, 2009; Van Overwalle, 2009, 2010; Kesselheim, 2010).

In Europe, the four essential pre-conditions governing the patentability of inventions under

the European Patent Convention (EPC) are laid down in Art. 52(1) EPC, which reads: 'European patents shall be granted for any *inventions*, in all fields of technology, provided that they are new, involve an inventive step and are susceptible of industrial application'. Thus, in a first step, in order to be patentable, there needs to be an invention. Thereafter, that invention has to fulfil the patentability requirements of novelty, inventive step and industrial applicability. The concept of 'invention' as such is not defined in the EPC, but the Implementing Regulations to the EPC do specify that the invention must have a technical character (Rule 29(1)), that is related to a technical field (Rule 27(1)(a)) and concerned with a technical problem (Rule 27(1)(c)). It is clear from these rules that 'technicality' is a key precondition for qualification as a patentable invention in Europe.

Article 52(2) EPC lists exclusions which should not be regarded as inventions, if claimed 'as such' (Article 52(3) EPC), because they are abstract in nature (*discoveries*) or non-technical in nature (scientific theories or methods for performing mental acts).

Thus in Europe, whether natural phage and cocktails of natural phage are to be regarded as *inventions* eligible for patent protection, or whether they are *discoveries* or principles of nature and thus excluded from patentability, depends on the technical character related to the claimed subject matter. There must be something more than mere disclosure of a natural phenomenon. Statutory, any 'biological material isolated from its natural environment or produced by means of a technical process, may be the subject of an invention even if it previously occurred in nature' (Art. 3 Biotechnology Directive 98/44/EC). Thus, in principle, a naturally occurring phage might become patentable as soon as some human intervention is needed to isolate the phage from its natural environment by any technical means as long as the phage can be properly characterized by either the process by which it is obtained (product-by-process claim), by its structure or by other means. Even so, cocktails of naturally occurring phage might be patentable as a result of a technical contribution.

In the US patent law Code (U. C), general requirements for patentability are listed in the 35 USC §101. That article defines that subject matter may be patentable, provided it or its improvement (1) belongs to one of four distinct classes, namely a process, machine, manufacture, or composition of matter, that is (2) new and (3) useful and (4) non-obvious (§103). A precondition for patentability as to its 'technical character' does not seem to be explicitly present in US patent law. Even though the US does not have a statutory counterpart to Article 52(2) EPC, exceptions to patentability are established by case law and certain categories are also excluded from patentability, such as products of nature, laws of nature and/or natural phenomena, and abstract ideas or basic human knowledge or thought. But what exactly means 'abstract ideas or basic human knowledge or thought'?

The US case law is clear on the aspect of non-naturally occurring organisms.

Most notably, the Supreme Court addressed the question in the 1980 landmark case *Diamond v. Chakrabarty*, 447 U.S. 303 considering a live, human-made genetically modified bacterium as patentable subject matter, because the newly formed bacterium had markedly different characteristics from any found in nature. According to the Court, 'a mere purification of known materials does not result in a patentable product, unless the product obtained has properties and characteristics which were different in kind from those of the known product rather than in degree'. The *Chakrabarty* case has been viewed as mandating the patentability of non-naturally occurring, non-human multicellular organisms such as transgenic animals, genetic materials, and purified biologically produced compounds such as enzymes.

For naturally occurring organisms, the US case law has also been developed in a specific way.

In 1948 the Supreme Court of the US considered a patent case known and recorded as Funk Brothers Seed Co. V. Kalo Inoculant Co. 333 U.S. 127 related to a mixture of several naturally occurring species of nitrogen-fixing bacteria. The Court held that the qualities of these bacteria are the work of nature and hence these qualities are not patentable.

However, other cases in the US Court of Customs and Patent Appeals (CCPA, which was renamed in 1982 as the Court of Appeals for the Federal Circuits) such as the 1977 *In re Application*

of Bergy, 563 F.2d 1031 (CCPA) and the 1979 *In re Kratz*,592 F.2d 1169 (CCPA) have provided some support for the patentability of various biological materials, including cells, proteins, and organisms if isolated or purified from their natural environment or pre-existing material. On the other hand, recently, the ruling in the US Court for the Southern District of New York by Judge Robert Sweet in a case between the *Association of Molecular Pathology (AMP)* and the *US Patent and Trademark Office (USPTO)* invalidated seven patents claiming genes and genetic diagnostic methods held by Myriad Genetics (Huys *et al.*, 2009; Akst, 2010; Cho, 2010; Huys *et al.*, 2011). Very recently however a new US court decision reversed Myriad gene patent ruling for *BRCA1* and *BRCA2* (Brower, 2011).

Thus the discussion is still ongoing and shows that the topic is hotly debated and not yet set.

Although related to naturally occurring genes, this decision restarted the discussion about the patentability for genes, but also for other types of naturally occurring biological (lifelike) entities, such as phage, and organisms. A similar situation is found in another medical therapeutic situation, namely the Faecal Microbiota Transplantation (FMT) intervention for diseases such as pseudomembranous colitis due to *Clostridium difficile* and inflammatory bowel disease (IBD) including Crohn's syndrome. Indeed bacterio-transplantation therapy faces analogous hurdles of IP and regulatory classification as phage therapy before being recognized and introduced in routine medical practices (McKenna, 2011; Landy *et al.*, 2011).

It is a very thin line that separates *inventions* from *discoveries* under both US law and European law. Therefore, it is very useful to investigate the second very important patentability requirement, namely novelty, which is of high relevance for naturally occurring phage. An invention can be patented only if it is new. An invention is considered to be new if it does not form part of the state of the art. European case law established that a natural substance which has been isolated for the first time and which had no previously recognised existence, does not lack novelty because it has always been present in nature as was the case for the Howard Florey Institute's Application on Relaxin OJEPO 1995, 388 (V 0008/94). In other words, this means that for a phage or the cocktail of phages as claimed in a patent, it should never have been isolated or produced before. Scientific literature with respect to phage as natural source to treat human bacterial infections exists and is increasing as mentioned in the introduction. In addition some clinical studies and reports on humans, using phage and performed in the Eastern part of Europe, have recently been translated into English (Chanishvili, 2009). Therefore, many phage and their uses have been disclosed over the past decades to almost a century. European law allows the patentability of known substances (as phage might be) if claimed for use in a medical method, provided that such use is new, meaning that such use may not be comprised in the state of the art (first medical use claim). We have to keep in mind that for novelty, the US applies a different 'state of the art' as the EU. In the US, the invention is considered as being part of the state of the art (hence not patentable) if it was known or used by others only in the US itself, or if it is patented or described in a printed publication in the US or outside the US. Thus in case such isolated phage are known in another country, it can still be patentable in the US.

In the past, patents on purified natural products of many kinds have been granted. For instance, in 1873, Louis Pasteur was granted US141072 patent for 'Yeast, free from organic germs of disease, as an article of manufacture'. And in 1903 Takamine obtained US730176 patent for adrenaline purified from gland tissue. More recently, in the field of phage applications, several patents for phage used in the food sector were granted, such as US7507571 (food additive) owned by Intralytix, Inc., claiming 'an isolated phage of a phage strain selected from a [specific] group, [somewhere] deposited under a [specific] accession number, together with variants thereof, wherein said variants retain the phenotypic characteristics of said deposited phage and wherein said phage, and variants thereof, have lytic activity against *Listeria monocytogenes* strains'. More important for therapeutic use is the US patent 7459272 of Intralytix, Inc., claiming 'a method for reducing the risk of bacterial infection or sepsis in a person colonized with pathogenic bacteria comprising treating the colonized person

with a pharmaceutical composition containing phage of one or more strains which produce lytic infections in said pathogenic bacteria'. In 2001, a European patent application (EP1250143 A2) was filed claiming 'a method for reducing the risk of bacterial infection or sepsis in a susceptible patient by treating the susceptible patient with a pharmaceutical composition containing phage of one or more strains which produce lytic infections in pathogenic bacteria'. Such claim was considered not to be novel. The applicant tried to rescue the claim by using a second medical use wording, however, in 2004, this application was deemed to be withdrawn. Only recently 'a method for production of compositions of phage with a specific titre and total yield' was claimed in the US by Phage Biopharm LLC (US7588929). No European counterpart has been published yet. Two other interesting patents are the US patent 7758856 and US patent 7807149, both of Biocontrol Limited and granted in 2010. They claim 'a composition for treating a bacterial biofilm', as well as 'a method for treating a biofilm infection' and 'a bacteriophage containing therapeutic agents'. Those 'patents' however, when analysed in depth do not patent phage as such, but a whole process from which phage is a part. They also are very broad in definition. One might ask what the real value of such a patent is in practical terms? A similar patent, covering also the use of phage for the treatment of biofilms, has been granted in Europe under the EPI code EP1587520 B1 and is owned by the Health Protection Agency (GB).

In summary, diverging views across Europe and the US exist on patenting biological material. Aside from the requirements of novelty, inventive step and industrial applicability (that are the same for Europe and the US), in order to be patentable in Europe, some technical intervention is needed to isolate the phage from its natural environment and the phage needs to be properly characterized. But this 'technical intervention' has been basically known since d'Hérelle while the fact that the phage 'needs to be well characterized' seems obvious and technically not a big problem (Merabishvili *et al.*, 2009). In the US, the phage, as claimed in a patent, needs to have markedly different characteristics from any found in nature. However, if we talk about naturally occurring, exclusively lytic phage, which are our object of concern here, then they remain as they are found in nature. It is only when working with genetically modified phage that we can agree with the US statement. The use of 'manipulated or engineered phage' has certainly applications, which could be patented, but, considering the current concerns about potential risks for public health and the environment which may arise from genetically modified organisms (GMOs), they are not likely to be given more easily a market licensing approval in the near future even if a smoother regulatory pathway was constructed. Phage-derived products (e.g. cell wall-degrading enzymes such as endolysins) can and probably will be licensed and marketed within a few years. They may also select resistance, but presumably at a lower rate than antibiotics. Of course, these phage-derived products lack the capacity of self-replication and adaptation in the infectious site.

In this chapter we focus on natural lytic phage just because of their natural intrinsic bacterial co-evolutionary aspect allowing a sustainable antibacterial treatment approach that is suitable for quick, flexible, and potentially cheaper therapeutic applications.

Patents claiming genes or natural entities like plants and, for instance, phage seem difficult or even (and perhaps correctly) 'impossible'. Even 'inventing around' as Van Overwalle (2010) writes is not easy and therefore Taubman (2009) thinks that 'technology specific interventions' are required. From an industrial point of view this is difficult to accept, but this is mainly because we are still fixed on the classical thinking paths. As biologists we should know that nothing is stable and as a consequence our 'laws' and 'attitudes' should adapt and co-evolve.

At a concrete level, however, research groups are trying to propose constructive solutions such as the establishment of 'genetic pools', 'clearing houses', 'patent pool', 'alternative licensing models' amongst others (Taubman, 2009; Van Overwalle, 2009). They are trying to work out the organization of several types of interface institutions such as clearing houses in order to facilitate the access to patents and technologies by research exemptions, the creation of patent pools or 'collaborative patent systems' for affordable

redistribution among interested developers. This matter was recently well reviewed by Van Zimmeren *et al.* (2011).

The renewed interest in natural phage as therapeutic agents might trigger scientists and entrepreneurs' creativity in defining the contours of appropriate patent claims for phage. Such new ideas on patentability should not be based on the existing classical model but on a broader 'new' philosophy in relation to sustainable economic and industrial development. Thinkers such as Ricardo Petrella (and his colleagues from the Group of Lisbon) and Jeffrey D. Sachs are leading this potential paradigm shift (The Group of Lisbon, 1995; Sachs, 2008). Petrella claims that today 'being competitive' is no longer a tool for increased development but an aim on itself. This increasingly implies, that the possession of patents, often as a strategic weapon, is thought to be more important (in a short-term perspective) than owning a truly functional innovative technology. This kind of attitude tends to block the development of new biomedical approaches. The patent tragedy is indeed exemplified by the millions of AIDS victims that die while treatment drugs exist and raises deep questions about global intellectual property rights and Western Ethics. How can the benefits of a global patent system that provides incentives for innovation and continuous development be combined with an assurance that the targeted people (rich and poor) gain access to the medical care that they need and have right to? As mentioned earlier the patentability of phage or phage-related procedures is key, but thorny, for the development of the potential of this rediscovered tool, which is urgently needed in an increasingly antibiotic resistant world (Levy and Marshal, 2004; Thiel, 2004; Kümmerer and Henninger, 2003; Kümmerer, 2004; Pirnay *et al.*, 2005; Kumarasamy, 2010; Abraham, 2011; Cooper and Shlaes, 2011). In our opinion, changes to the patents, patentability and restrictive licensing, common in the current pharmaceutical environment are essential for our global medical future. This need for change, after an analysis of the multifactorial aspects of antibacterial resistance, with a focus on our current Western societal profitable attitudes brings us to a 'medicine^{TM}' situation which we, amongst others, discussed already previously (Pirnay *et al.*, 2003b). This 'medicine^{TM}' situation is the consequence of the current industrialization of medicine, the 'medical industry', which neglects the fact that biological systems do not behave and consequently do not respond to markets as expected for typical mechanical behaving systems. Medicine, basically an applied biological science, based on biology a fundamental science on its own, is governed by inherent different rules than classic mechanics, and consequently should be considered as a common public good.

Along this line of thinking, the Group of Lisbon, led by Ricardo Petrella, proposes an evolution to world cooperative governance, which is based on a global contract that requires that each decision must be linked to the fact that each person should have access to all basic livelihoods, including health access, which actually is often blocked by our out-dated economic model. This is also the result of the analysis of Selgelid in his well documented article 'Ethics and drug resistance' who concludes that 'public goods warrant special treatment among which governmental intervention/funding will play a key role' (Selgelid, 2007). In this context, a 'tailor-made' approach as proposed and discussed by Pirnay and colleagues for phage therapy could eventually be developed under the umbrella of, for example, the WHO (Pirnay *et al.*, 2010; Pirnay *et al.*, 2012). The WHO recognizes the importance of the worldwide antibiotic resistance issues and is discussing new incentives to push the pharmaceutical industry in alliance with governments to launch new antimicrobial drug research and development projects (http://www.who.int/drugresistance/en/). Specific working groups were created such as the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) and the Intergovernmental Task Force on Antimicrobial Resistance (ITFAR). The 2011 World Health Day (7 April 2011) was specifically dedicated to the antimicrobial resistance issue. The strong eye catching slogan was as follows: '*Antimicrobial resistance: no action today, no cure tomorrow*' (http://www.who.int/world-health-day/2011/en/). We are therefore surprised that, although an awareness of the problem exists, 'phage therapy' is, as far as

we are aware, still not mentioned by this organization as one of the potential options in the fight against bacterial infections.

Conclusions

Having analysed several major aspects (negatively) influencing the reintroduction of phage therapy as a potential antibacterial treatment we have to realise that Ethics, Science/Technology and Economy are intertwined and form a field of tension which can result in conflicting, although eventually constructive, situations.

Medicinal product regulation and marketing models evolved to predominantly accommodate stable, precautionary and profitable drugs with sufficient IP protection. Unfortunately, sustainable phage therapy seems to lack strong IP protection, which hampers its re-introduction in practical medicine today and justifiably raises ethical discussions.

In this respect we can ask ourselves 'is our current drug development and marketing model still the best suited and ethically consistent?' We live in a globalizing society with a growing awareness of evolution and sustainability. This should also trickle down into the field of drug development. Are the current overregulation and IP issues ethically tolerable in a time when increasing numbers of patients die as a consequence of antibiotic resistant infections?

The current (European) regulatory setting allows for eventual sporadic clinical trials under the responsibility and supervision of leading Medical Ethical Committees and/or borderline applications under the umbrella of the Declaration of Helsinki. Some strategies, for example the strategy adopted in Poland are possible but they will not enable the further optimal development of phage therapy in clinical practice at large. This is due to the difficulty of setting up well designed large scale and/or multi-centric (European) studies in order to prove the efficacy of this approach. Also will there be a need for studies which could optimize this therapeutic antibacterial approach in the different medical application fields (especially for such aspects as for example the most appropriate galenic formulation, the application frequency, the optimal phage concentration amongst other essential clinical parameters). There is a clear need for an adapted European regulation tailored to phage therapy, allowing the justified and broad therapeutic use of phage as an alternative or complement to antibiotics. In order to make full and safe use of phage therapy the frame has to allow, beside a 'prêt-à-porter' development a real flexible near the patient 'sur-mesure' approach, the latter being intrinsically the better suited of both models.

We support a long-term solution, such as the creation of a specific section for phage therapy under the actual Advanced Therapy Medicinal Product (ATMP) Regulation (EC) No 1394/2007, amending Directive 2001/83/EC and Regulation (EC) No 726/2004. This Regulation presently includes products for Gene Therapy, and for Somatic Cell Therapy and Tissue Engineered Products. We propose the creation of a fourth section, named Bacteriophage Therapy Preparations, which could be divided into two distinct sections: 'tailor-made use in a hospital environment' (via a specific hospital exemption regulation) versus 'industrially produced for market placement' (Verbeken *et al.*, 2007; Pirnay *et al.*, 2010). Amending Commission Directive 2003/63/EC could also be considered. Placing Bacteriophage Therapy Preparations under or next to the current sections foreseen for Biological Medicinal Products or Particular Medicinal Products could also be a possible approach.

A major hurdle is the lack of societal realization that the classic economic, ethical, medical and scientific ways of thinking have to be adapted to our natural environment, which is a world in constant movement and evolution. Patent related issues should be seen from a more societal impact perspective and not only from the pure straight-line economic point of view, currently at the forefront. Our ethical views also have to be re-evaluated if we prevent patients' access to therapy, which has at least empirically proven to be efficacious, under the umbrella of 'patient's safety'. The role of fundamental scientists and medical practitioners is also to show new insights and to advocate for some change, especially when the current societal views, often too reductionist and based upon short term considerations, are blocking a further promising development.

This view of the future of 'Phage Therapy' was described in a primary opinion paper by Pirnay and colleagues (Pirnay *et al.*, 2010) and further discussed in two recent review articles (Pirnay *et al.*, 2012; Verbeken *et al.*, 2012). During a recent EMBO sponsored meeting on 'Viruses of microbes' organized in Brussels at the Royal Military Academy a whole workshop and debate session was dedicated to the issue (Brüssow, 2012). Phage therapy and antibiotic therapy, as well as the two proposed developmental models, should not be mutually exclusive as also discussed by Maura and Debarbieux (Maura and Debarbieux, 2011). At the contrary some studies even argue for a synergistic effect of phages and antibiotics as well as a limitation of the emergence of antibiotic resistance as a result of using phages (Comeau *et al.*, 2007; Zhang and Buckling, 2011; Ryan *et al.*, 2012; Kirby, 2012).

On 14 February 2011 the question 'What is the European View on the status of Phage as antibacterial agent,' was officially asked by the Belgian Christian Democrat Ivo Belet and his French parliamentarian colleague from the Socialist fraction Catherine Trautmann to the European Commission (http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+WQ+E-2011-001144+0+DOC+XML+V0//EN&language=CS). This shows that reflections on the idea of reintroducing phage therapy diffuses out of the world of biomedical science and the pharmaceutical industry to the political arena as a topic for public discussion in a time of antibiotic shortages. The official answer, received on 29 March 2011 was formulated as follows: 'The Commission considers that the existing regulatory framework is adequate for bacteriophage therapy without the need for an extra set of documentation for bacteriophage therapy' (http://www.europarl.europa.eu/sides/getAllAnswers.do?reference=E-2011–001144&language=CS). Thus the official answer was, as expected, that phage is covered by the actual European medicinal product regulation. This official statement was our starting point for opening discussions, as described, at the level of the European Medicines Agency and its specifically dedicated Innovation Task Force (ITF) unit. Those discussions are still going on and will hopefully result in an adequate change enabling the implementation of phage therapy in clinical practice.

Acknowledgements

We would like to thank Mr William Anderson for reviewing the text for language. We would also like to thank Jérôme Larché, MD, MSc and Head of the Intensive Care Unit, Hospital of Narbonne, France. As president of PhagEspoirs, a non-profit organization aiming at promoting research on phage, he reviewed and commented actively this document. We also would like to thank Geert Laire, MD, Surgeon General and Chief of the Belgian Medical Defence Component, Pierre Neirinckx, MD, Colonel and Director of the Queen Astrid Military Hospital and Serge Jennes, MD, Lt. Colonel and Head of the Burn Wound Centre of the Queen Astrid Military Hospital for their continuous support to our phage therapy project. And last but not least we would also like to thank Valérie De Vos, for the multiple reflections and discussions on traditional medical systems versus our current medical industry, based on her African cultural anthropological background and her crisis management approach in conflicting fields of globalization.

Web resources

- WHO. Policy Package to Combat Antimicrobial Resistance. http://www.who.int/bulletin/volumes/89/5/11-088435/en/ (accessed July 2013).
- WMA Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net/en/30publications/10policies/b3/index.html.pdf?print-media-type&footer-right=[page][toPage (accessed July 2013).

References

Abedon, S.T., Kuhl, S.J., Blasdel, B.G., and Kutter, E.M. (2011). Phage treatments of human infections. Bacteriophage. *1*, 66–85.

Agarwal, K.N., and Bhasin, S.K. (2002). Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations Actimel and Indian Dahi. Eur. J. Clin. Nutr. *56(S4)*, 556–559.

Aiello, A.E., King, N.B., and Foxman, B. (2006). Ethical conflicts in public health research and practice. Am. J. Public Health. *96*, 1910–1914.

Akst, J. (2010). Key cancer patents killed. http://www.the-scientist.com/blog/display/57265/ (accessed19/10/10), part of The Scientist.http://www.the-scientist.com/(accessed on 19/10/10).

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., and Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. *8*, 251–259.

Association for Molecular Pathology. (2010). U.S. patent and trademark office, Southern District of New York (29 March 2010).

Bamford, D.H. (2003). Do viruses form lineages across different domains of life? Res. Microbiol. *154*, 231–236.

Baquero, F., Coque, T.M., and Canton, R. (2003). Antibiotics, complexity and evolution. ASM News. *69*, 547–552.

Barr, J.J., Auro, R., Furlan, M., Whiteson, K.L., Erb, M.L., Pogliaéno, J., Stotland, A., Wolkowicz, R., Cutting, A.S., Doran, K.S., *et al.* (2013). Bacteriophage adhering to mucus provide a non-host-derived immunity. Proc. Natl. Acad. Sci. U.S.A. *110*, 10771–10776.

Bartoloni, A., Pallechi, L., Rodriguez, H., Fernandez, C., Mantella, A., Bartalesi, F., Strohmeyer, M., Kristiansson, C., Gotuzzo, E., Paradisi, F., *et al.* (2009). Antibiotic resistance in a very remote Amazonas community. Int. J. Antimicrob. Agents. *33*, 125–129.

Beauchamp, T.L., and Childress, J.F. (2009). Principles of biomedical ethics (New York/Oxford, Oxford University Press).

Bell, P.J.L. (2001). Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus? J. Mol. Evol. *53*, 251–256.

Benner, S.A. (2010). Defining life. Astrobiology *10*, 1021–1030.

Bergh, O., Borsheim, K.Y., Bratbak, G., and Heldal, M. (1989). High abundance of viruses found in aquatic environments. Nature *340*, 476–468.

Berman, S.H., Eichelsdoerfer, P., Yim, D., Elmer, G.W., and Wenner, C.A. (2006). Daily ingestion of a nutritional probiotic supplement enhances innate immune function in healthy adults. Nutr. Res. *26*, 454–459.

Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A.N., Powell, B., Carlton, R., and Merril, C.R. (2002). Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect. Immun. *70*, 204–210.

Bošnjak, S. (2001). The Declaration of Helsinki – the cornerstone of research ethics. Arch. Oncol. *9*, 179–184.

Brower, V. (2012). US court reverses gene patent ruling for *BRCA1* and *BRC2*. Lancet Oncol. *12*, 835.

Brüssow, H. (2005). Phage therapy: the *Escherichia coli* experience. *151*, 2133–2140.

Brüssow, H. (2009). The not so universal tree of life or the place of viruses in the living world. Phil. Trans. R. Soc. B *364*, 2263–2274.

Brüsssow, H. (2012). What is needed for phage therapy to become a reality in Western medicine? *434*, 138–142.

Bruttin, A., and Brüssow, H. (2005). Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob. Agents and Chemother. *49*, 2874–2878.

Brzuszkiewicz, E., Thurmer, A., Schuldes, J., Leinbach, A., Lieregang, H., Meyer, F.D., Boelter, J., Petersen, H., Gottschalk, G., and Daniel, R. (2011). Genome sequence analyses of two isolates from the recent *Eschericha coli* outbreak in Germany reveal the emergence of a new pathogen type Entero-Aggregative-Haemorhagic-*Escherichia coli* (EAHEC). Arch. Microbiol. *193*, 883–891.

Buckling, A., and Rainey, P.B. (2002). Antagonistic coevolution between a bacterium and a bacteriophage. Proc. Biol. Sci. *269*, 931–936.

Burdock, G.A., and Carabin, I.G. (2004). Generally recognized as safe (GRAS): history and description. Toxicol. Lett. *150*, 3–18.

Bush, K., Courvalin, P., Dantas, G., Davies, J., Eisenstein, B., Huovinen, P., Jacoby, G.A., Kishony, R., Kreiswirth, B.N., Kutter, E., *et al.* (2011). Tackling antibiotic resistance. Nat. Rev. Microbiol. *9*, 894–896.

Chanishvili, N. (2009). A Literature Review of the Practical Application of Bacteriophage Research (Tbilisi, Georgia, UK HPA, Eliava Institute of Bacteriophage, Microbiology and Virology).

Chanishvili, N. (2012). Phage therapy – history from Twort and d'Hérelle through Soviet experience to current approaches. Adv. Virus Res. *83*, 3–40.

Cherniak, E.P. (2010). Bugs as drugs, Part 1: Insects. The 'New' alternative Medicine for the 21st century. Altern. Med. Rev. *15*, 124–135.

Chibani-Chenouffi, S., Sidoti, J., Brüttin, A., Kutter, E., Sarker, S., and Brüssow, H. (2004). *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. Antimicrob. Agents Chemother. *48*, 2558–2569.

Cho, M. (2010). Patently unpatentable: implications of the Myriad court decision on genetic diagnostics. Trends Biotechnol. *28*, 548–551.

Cleland, C.E. (2007). Epistemological issues in the study of microbial life: alternative terran biospheres? Stud. Hist. Phil. Biol. & Biomed. Sci. *38*, 847–861.

Comeau, A.M., Tétart, F., Trojet, S.N., Prère, M.F., and Krish, H.M. (2007). Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. PLoS One *2*, e799.

Cooper, M.A., and Shlaes, D. (2011). Fix the antibiotics pipeline. Nature *472*, 32.

Corbellini, G. (2008). Evolution based medicine (EBM), an epistemological framework for thinking of, and dealing with the so called « crisis of medicine ». J. Hist. Med. *20*, 115–139.

Darwin, C. (1968). The Origin of Species. London, UK, Penguin Classics, Penguin Group.

D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G.B., Poinar, H.N., and Wright, G.D. (2011). Antibiotic resistance is ancient. Nature *477*, 457–461.

D'Costa, V.M., McGrann, K.M., Hughes, D.W., and Wright, G.D. (2006). Sampling the antibiotic resistome. Science *311*, 374–377.

Dabrowska, K., Switala-Jelen, K., Opolski, A., Weber-Dabrowska, B., and Gorski, A. (2003). Bacteriophage penetration in vertebrates. J. Appl. Microbiol. *98*, 7–13.

Debarbieux, L., Leduc, D., Maura, D., Morello, E., Criscuolo, A., Grossi, O., Balloy, V., and Touqui, L. (2010). Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. J. Infect. Dis. *201*, 1096–1104.

Dublanchet, A. (2009). Des virus pour combattre les infections. Favre, Lausanne, Switzerland.

European Parliament. (2003). Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and the council of the Community code relating to medicinal products for human use. Official Journal of the European Union (translated into Belgian Law by Royal Decree of 04.03.2004, published in the Belgian Official Journal on 10.03.2004).

European Parliament. (2004). Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official journal of the European Communities (translated into Belgian Law by Royal Decree of 04.03.2004, published in the Belgian Official Journal on 10.03.2004).

Faruque, S.M., Naser, I.B., Islam, M.J., Faruque, A.S.G., Ghosh, A.N., Nair, G.B., Sack, D.A., and Mekalanos, J.J. (2005a). Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proc. Natl. Acad. Sci. U.S.A. *102*, 1701–1707.

Faruque, S.M., Islam, M.J., Ahmad, Q.S., Faruque, A.S.G., Sack, D.A., Nair, G.B., and Mekalanos, J.J. (2005b). Self-limiting nature of seasonal cholera epidemics: role of host-mediated amplification of phage. Proc. Natl. Acad. Sci. U.S.A. *102*, 6119–6124.

FDA (2006). FDA 21 CFR Part 172: Food Additives Permitted for Direct Addition to Food for Human Consumption; Bacteriophage Preparation. Federal Register, Rules and Regulations No. 160. *71*, 47729–47732.

Forterre, P. (2001). Genomics and early cellular evolution, the origin of the DNA world. Life Sci. *324*, 1067–1076.

Forterre, P. (2006). The origin of viruses and their possible role in major evolutionary transitions. Virus Res. *117*, 5–16.

Fuhrman, J.A. (1999). Marine viruses and their biogeochemical and ecological effects. Nature *399*, 541–548.

Gilmore, B.F. (2012). Bacteriophages as anti-infective agents: recent developments and regulatory challenges. Expert. Rev. Anti Infect. Ther. *10*, 533–535.

Gold, E.R., Kaplan, W., Orbinski, J., Harland-Logan, S., and N-Marandi, S. (2009). Are patents impeding medical care and innovation? PLoS Medicine *7*, e1000208.

Gorski, A., Miedzybrodzki, R., Borysowski, J., Weber-Dabrowska, B., Lobocka, M., Fortuna, W., Letkiewiecz, S., Zimecki, M., and Filby, G. (2009). Bacteriophage therapy for the treatment of infections. Curr. Opin. Investig. Drugs *10*, 766–774.

Hall, A.R., De Vos, D., Friman, V.P., Pirnay, J.P., and Buckling, A. (2012). Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa in vitro* and in Wax Moth larvae. Appl. Environment. Microbiol. *78*, 5646–5652.

Hendrix, R.W. (2002). Bacteriophages: evolution of the majority. Theor. Popul. Biol. *61*, 471–480.

d'Hérelle, F. (1917). Sur un microbe invisible antagoniste des bactéries dysentériques. C.R. Acad. Sci. *165*, 373–375.

d'Hérelle, F. (1919). Sur le rôle du microbe bacteriophage dans la typhose aviaire. C.R. Acad. Sci. *169*, 932–934.

Human, D., and Fluss, S.S. (2001). The World Medical Association's Declaration of Helsinki: historical and contemporary perspectives. Available at: http://www.wma.net

Huys, I., Berthels, N., Matthijs, G., and Van Overwalle, G. (2009). Legal uncertainty in area of genetic diagnostic testing. Nat. Biotechnol. *27*, 903.

Huys, I., Van Overwalle, G., and Matthijs, G. (2011). Gene and genetic diagnostic method patent claims: a comparison under current European and US patent law. Eur. J. Hum. Genet. *19*, 1104–1107.

Kapczynski, A. (2009). Innovation policy for a New Era. J. Law Med. Ethics *37*, 264–268.

Kesselheim, A.S. (2010). Using market-exclusivity incentives to promote pharmaceutical innovation. N. Engl. J. Med. *363*, 1855–1862.

Khawaldeh, A., Morales, S., Dillon, B., Alavidze, Z., Ginn, A.N., Thomas, L., Chapman, S.J., Dublanchet, A., Smithyman, A., and Iredell, J.R. (2011). Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. J. Med. Microbiol. *60*, 1697–1700.

Kirby, A.E. (2012). Synergistic action of gentamicin and bacteriophage in a continous culture population of *Staphylococcus aureus*. PLoS One *7*, e51017.

Kumarasamy, K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S., *et al.* (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis. *10*, 597–602.

Kümmerer, K. (2004). Resistance in the environment. J. Antimicrob. Chemother. *54*, 311–320.

Kümmerer, K., and Henninger, A. (2003). Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin. Microbiol. Infect. *9*, 1203–1214.

Kutateladze, M., and Adamia, R. (2010). Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol. *28*, 591–595.

Kutter, E., De Vos, D., Gvasalia, G., Alavidze, Z., Gogokhia, L., Kuhl, S., and Abedon, S.T. (2010). Phage therapy in clinical practice: treatment of human infections. Curr. Pharm. Biotechnol. *11*, 69–86.

Lander, E.S., Linton, L.M., Birren, *et al.* (the international human genome consortium) (2001). Initial sequencing and analysis of the human genome. Nature *409*, 860–921.

Landy, J., Al-Hassi, H.O., McLaughlin, S.D., Walker, A.W., Ciclitira, P.J., Nicholls, R.J., Clark, S.K., and Hart, A.L. (2011). Review article: faecal transplantation therapy for gastrointestinal disease. Aliment. Pharmacol. Ther. *34*, 409–415.

Leibovici, L., Paul, M., and Ezra, O. (2012). Ethical dilemmas in antibiotic treatment. J. Antimicrob. Chemother. *67*, 12–16.

Levy, S.B., and Marshal, B. (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. *10*, S122-S129.

McCallin, S., Alam Sarker, S., Barretto, C., Sultana, S., Berger, B., Huq, S., Krause, L., Bibiloni, R., Schmitt, B., Reuteler, G., *et al.* (2013). Safety analysis of a Russian phage cocktail: From MetaGenomic analysis to oral application in healthy human subjects. Virology *443*, 187–196.

McKenna, M. (2011). Swapping germs: should fecal transplants become routine for debilitating diarrhea? Sci. Am. *305*, 34.

McVay, C.S., Velasquez, M., and Fralick, J.A. (2007). Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. Antimicrob. Agents Chemother. *51*, 1934–1938.

Mahony, J., McAuliffe, O., Ross, R.P., and van Sinderen, D. (2011). Bacteriophages as biocontrol agents of food pathogens. Curr. Opin. Biotechnol. *22*, 157–163.

Martinez, J.L., and Baquero, F.(2002). Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin. Microbiol. Rev. *15*, 647–679.

Marza, J.A., Soothill, J.S., Boydell, P., and Collyns, T.A. (2006). Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. Burns *32*, 644–646.

Maura, D., and Debarbieux, L. (2011). Bacteriophages as twenty-first century antibacterial tools for food and medicine. Appl. Microbiol. Biotechnol. *90*, 851–859.

Mayr, E. (2004). What makes biology unique? Considerations on the autonomy of a discipline. Cambridge University Press.

Merabishvili, M., Pirnay, J.-P., Verbeken, G., Chanishvili, N., Tediashvili, M., Lashkhi, N., Gionti, T., Krylov, V., Mast, J., Van Parijs, L., *et al.* (2009). Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One *4*, e4944.

Merabishvili, M., De Vos, D., Verbeken, G., Kropinski, A.M., Vandenheuvel, D., Lavigne, R., Wattiau, P., Mast, J., Ragimbeau, C., Mossong, J., *et al.* (2012). Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. PLoS One, *7*, e52709.

Mi, S., Lee, X., Li, X.-P., Veldman, G., Finnerty, H., Racie, L., LaVilie, E., Tang, X.-Y., Edouard, P., Howes, S., *et al.* (2000). Syncitin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature *403*, 785–789.

Międzybrodzki, R., Fortuna, W., Weber-Dąbrowska, B., and Górski, A. (2007). Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. Post. Med. Hig. Dośw. *61*, 461–465.

Miedzybrodzki, R., Borysowski, J., Weber-Dabrowska, B., Fortuna, W., Letkiewicz, S., Szufnarowski, K., Pawelczyk, Z., Rogoz, P., Klak, M., Wojtasik, E., *et al.* (2012). Clinical aspects of phage therapy. Adv. Virus Res. *83*, 73–119.

Morello, E., Saussereau, E., Maura, D., Huerre, M., Touqui, L., and Debarbieux, L. (2011). Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. PLoS One *6*, e16963.

Parracho, H.M., Burrowes, B.H., Enright, M.C., McConville, M.L., and Harper, D.R. (2012). The role of regulated clinical trials in the development of bacteriophage therapeutics. *6*, 279–286.

Pirnay, J.-P., De Vos, D., Cochez, C., Bilocq, F., Pirson, J., Struelens, M., Duinslaeger, L., Cornelis, P., Zizi, M., and Vanderkelen, A. (2003a). Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J. Clin. Microbiol. *41*, 1192–1202.

Pirnay, J.-P., De Vos, D., Zizi, M., and Heyman, P. (2003b). No easy way to exterminate 'superbugs' at the dawn of the third millennium. Exp. Rev. Anti-Infectiv. Ther. *1*, 523–525.

Pirnay, J.-P., Mathijs, S., Colak, H., Chablain, P., Bilocq, F., Van Eldere, J., De Vos, D., Zizi, M., Triest, L., and Cornelis, P. (2005). Global *Pseudomonas aeruginosa* biodiversity as reflected in a Belgian river. Env. Microbiol. *7*, 969–980.

Pirnay, J.-P., De Vos, D., Verbeken, G., Merabishvili, M., Chanishvili, N., Vaneechoutte, M., Zizi, M., Laire, G., Lavigne, R., Huys, I., *et al.* (2010). The Phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm. Res. *28*, 934–937.

Pirnay, J.-P., Verbeken, G., Rose, T., Jennes, S., Zizi, M., Huys, I., Lavigne, R., Merabishvili, M., Vaneechoutte, M., Buckling, A., and De Vos, D. (2012). Introducing yesterday's phage therapy in today's medicine. Future Virol. *7*, 379–390.

Raoult, D., and Forterre, P. (2008). Redefining viruses: lessons from the mimivirus. Nat. Rev. Microbiol. *6*, 315–319.

Reid, G. (2011). Quo vadis-EFSA? Benef. Microb. *2*, 177–179.

Rhoads, D.D., Wolcott, R.D., Kuskowski, M.A., Wolcott, B.M., Ward, L.S., and Sulakvelidze, A. (2009). Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J. Wound Care *18*, 237–243.

Rolain, J.M., Canton, R., and Cornaglia, G. (2012). Emergence of antibiotic resistance: need for a new paradigm. Clin. Microbiol. Infect. *18*, 615–616.

Ryan, E.M., Gorman, S.P., Donnelly, R.F., and Gilmore, B.F. (2011). Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy. J. Pharm. Pharmacol. *63*, 1253–1264.

Ryan, E.M., Alkawareek, M.Y., Donnelly, R.F., and Gilmore, B.F. (2012). Synergistic phage–antibiotic combinations for the control of *Escherichia coli* biofilms in vitro. FEMS Immunol. Med. Microbiol. *65*, 395–398.

Sachs, J.D. (2008). Common Wealth: Economics for a crowded Planet. Penguin, London, UK.

Sanders, M.E., and Huis in 't Veld, J. (1999). Bringing a probiotic-containing functional food to the market:

microbiological, product, regulatory and labeling issues. Antonie van Leeuwenhoek. *76*, 293–315.

Sarker, S.A., McCallin, S., Barretto, C., Berger, B., Pittet, A.C., Sultana, S., Krause, L., Huq, S., Bibiloni, R., Bruttin, A., *et al.* (2012). Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. Virology *434*, 222–232.

Schmieder, R., and Edwards, R. (2012). Insights into antibiotic resistance through metagenomic approaches. Future Microbiol. *7*, 73–89.

Selgelid, M.J. (2007). Ethics and drug resistance. Bioethics. *21*, 218–229.

Shanks, N., and Pyles, R.A. (2007). Evolution and medicine: the long reach of 'Dr. Darwin'. Philos. Ethics Humanit. Med. *2*, 4.

Shapiro, O.H., and Kushmaro, A. (2011). Bacteriophage ecology in environmental biotechnology processes. Curr. Opin. Biotechnol. *22*, 1–7.

Slopek, S., Weber-Dabrowska, B., Dabrowski, M., and Kucharewicz-Krukowska, A. (1987). Results of bacteriophage treatment of suppurative bacterial infections in the years 1981–1986. Arch. Immunol. Ther. Exp. *35*, 569–583.

Smith, H.W., and Huggins, M.B. (1982). Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol. *128*, 307–318.

Sulakvelidze, A. (2013). Using lytic bacteriophages to eliminate or significantly reduce contamination of food by food-borne bacterial pathogens. J. Sci. Food Agric. *93*, 3137–3146.

Sulakvelidze, A., Alavidze, Z., and Morris, J.G. Jr. (2001). Bacteriophage therapy. Antimicrob. Agents Chemother. *45*, 649–659.

Taubman, A. (2008). The international patent system and biomedical research: reconciling aspiration, policy and practice. AAPS J. *10*, 526–536.

Thiel, K. (2004). Old dogma, new tricks – 21st. century phage therapy. Nat. Biotechnol. *22*, 31–36.

Tirard, S., Morange, M., and Lazcano, A. (2010). The definition of life: a brief history of an elusive scientific endeavour. Astrobiology *10*, 1003–1009.

Tyebkhan, G. (2003). Declaration of Helsinki: the ethical cornerstone of human clinical research. Indian J. Dermatol. Venerol. Leprol. *69*, 245–247.

The Group of Lisbon. (1995). Limits to Competition. The MIT Press, Cambridge, MA, USA.

Valenti, R. (2012). Time, evolution and physical reductionism. EMBO Rep. *13*, 181–185.

Van Overwalle, G. (2009). Gene Patents and Collaborative Licensing Models, Patent Pools, Clearing Houses, Open Source Models and Liability Regimes (Cambridge: Cambridge University Press).

Van Overwalle, G. (2010). Turning patent swords into shares. Science *230*, 1630–1631.

Van Zimmeren, E., Vanneste, S., Matthijs, G., Vanhaverbeke, W., and Van Overwalle, G. (2011). Patent pools and clearing houses in the life sciences. *29*, 569–576.

Verbeken, G., De Vos, D., Vaneechoutte, M., Merabishvili, M., Zizi, M., and Pirnay, J.-P. (2007). European regulatory conundrum of phage therapy. Future Microbiol. *2*, 485–491.

Verbeken, G., Pirnay, J.-P., De Vos, D., Jennes, S., Zizi, M., Lavigne, R., Casteels, M., and Huys, I. (2012). Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine. Arch. Immunol. Ther. Exp. *60*, 161–172.

Villarreal, L.P. (2005). Overall issues of viruses and host evolution. In Viruses and the Evolution of Life, L.P. Villareal, ed. (Washington, DC, ASM press), pp. 1–28.

Villarreal, L.P., and Witzany, G. (2010). Viruses are essential agents within the roots and stem of the tree of life. J. Theor. Biol. *262*, 698–710.

Ward, P. (2005). Life As We Do Not Know It (New York, USA, Viking Penguin).

Weber-Dabrowska, B., Mulczyk, M., and Gorski, A. (2000). Bacteriophage therapy of bacterial infections: an update of our institute's experience. Arch. Immunol. Ther. Exp. *48*, 547–551.

Williams, P.D. (2010). Darwinian interventions: taming pathogens through evolutionary ecology. Trends Parasitol. *26*, 83–92.

Wills, Q.F., Kerrigan, C., and Soothill, J.S. (2005). Experimental bacteriophage protection against *Staphylococcus aureus* abscess in a rabbit model. Antimicrob. Agents. Chemother. *49*, 1220–1221.

Wommack, K.E., and Colwell, R.R. (2000). Virioplankton: viruses in aquatic ecosystems. Microbiol. Mol. Biol. Rev. *64*, 69–114.

World Medical Association. (2008). Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net/en/30publications/10policies/b3/index.html

Wright, A., Hawkins, C.H., Anggärd, E.E., and Harper, D.R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic resistant *Pseudomonas aeruginosa*: a preliminary report of efficacy. Clin. Otolaryngol. *34*, 349–357.

Zhang, Q.G., and Buckling, A. (2011). Phages limit the evolution of bacterial antibiotic resistance in experimental microcosms. Evol. Appl. *5*, 572–582.

2.1.2 European regulatory conundrum of bacteriophage therapy

G. Verbeken, D. De Vos, M. Vaneechoutte, M. Zizi, J.P. Pirnay

Future Microbiol. 2007; 2(5):485-491

International scientific journal, peer-reviewed

European regulatory conundrum of phage therapy

Gilbert Verbeken, Daniel De Vos†, Mario Vaneechoutte, Maya Merabishvili, Martin Zizi & Jean-Paul Pirnay

†Author for correspondence Hospital Center of the Base – Queen Astrid, Laboratory for Molecular & Cellular Technology, Burn Unit, Bruynstraat 1, B-1120 Neder-over-Heembeek, Belgium
Tel.: +32 2264 4856; Fax: +32 2264 4907; daniel.devos2@mil.be

The treatment of infectious diseases with antibiotics is becoming increasingly challenging. Very few new antimicrobials are in the pharmaceutical industry pipeline. One of the potential alternatives for antibiotics is phage therapy. Major obstacles for the clinical application of bacteriophages are a false perception of viruses as 'enemies of life' and the lack of a specific frame for phage therapy in the current Medicinal Product Regulation. Short-term borderline solutions under the responsibility of a Medical Ethical Committee and/or under the umbrella of the Declaration of Helsinki are emerging. As a long-term solution, however, we suggest the creation of a specific section for phage therapy under the Advanced Therapy Medicinal Product Regulation.

Keywords: advanced therapy, antibiotic resistance, bacteriophage, burn wound, Europe, phage therapy, probiotic, *Pseudomonas aeruginosa*, regulatory, *Staphylococcus aureus*, virus

The treatment of infectious diseases with antibiotics is becoming increasingly challenging. It often brings the clinician back in time to the pre-antibiotic era. Antibiotic resistance is a significant medical problem worldwide and alternatives are urgently needed [1–3]. The reasons are multiple and find their origin in disparate fields ranging from socioeconomics and cultural attitudes, to a lack of scientific and technological knowledge or inappropriate application of existing knowledge [4].

Very few new antimicrobials are in the pipeline of the pharmaceutical industry, which apparently focuses on other (more profitable) aspects of healthcare, such as impotence, and chronic diseases, such as hypertension and diabetes.

One of the potential alternatives for antibiotics is 'phage therapy'. Bacteriophages are among the most abundant and ubiquitous biological entities on earth. Lytic bacteriophages are the natural 'enemies' of bacteria. They are tiny (40-times smaller than a bacterium) often 'spider-like' creatures with a transparent box-shaped head (**Figure 1**). They attach to the walls of bacterial cells and subsequently inject their genetic material that is stored in the head. They take over the bacteria's genetic machinery, thereby forcing the bacteria to produce numerous copies of the bacteriophage inside itself causing it to explode (lyze), thus liberating vast numbers of new bacteriophages. Bacteriophages were discovered independently during World War I by the English microbiologist Frederick Twort and by the French–Canadian biologist Felix d'Herelle. D'Herelle announced that nature had provided humankind with a 'living', natural weapon against bacteria. However, bacteriophages proved to be a rather capricious therapy. The reason for this being that there are numerous types of bacteriophages, each killing only one specific variety (sometimes only one or a few strains) of bacteria. Since mixtures of bacteriophages were often used empirically – without efficient purification and without matching bacteriophages and bacteria – it is easy to understand why on some occasions the treatment was unsuccessful. This difficulty, and the advent of antibiotics, forced bacteriophages to the margins of Western medicine. Antibiotics are chemically well-defined and controllable substances, which exhibit an activity against a wide range of disease-causing bacteria (broad spectrum) and were thus more user-friendly and commercially more profitable than bacteriophages.

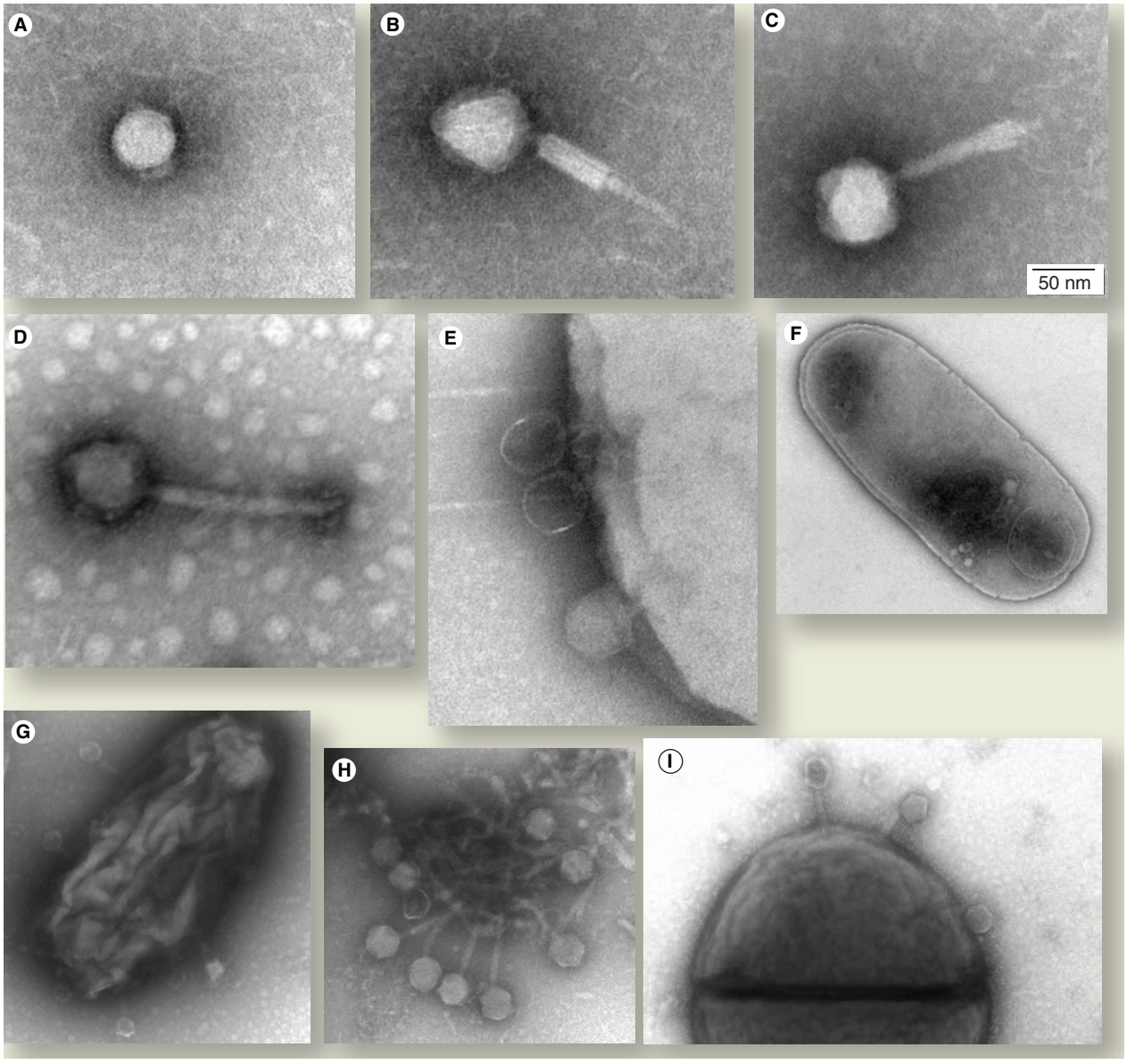
Today there is a renewed interest in the Western world for phage therapy. Modern molecular biology tools (e.g., rapid diagnostics, DNA-sequencing and fingerprinting) and purification techniques allow the development of pyrogen-free and well-characterized and targeted bacteriophage cocktails. However, there are a few economical and psychological obstacles that will have to be surmounted:

- The use of a virus to treat infectious diseases
- The use of a treatment coming from the former Soviet Union
- Patent issues

The increasing number of papers and books appearing on the subject, as well as the emergence of specifically dedicated companies, show that there is a real interest in the subject worldwide [5–6].

Another major problem is the regulatory conundrum. This paper will focus on the regulatory vacuum surrounding phage therapy in

Figure 1. Electron micrographs of the bacteriophages present in the bacteriophage cocktail that will be used in our Burn Wound Center.



(A) *Pseudomonas aeruginosa* bacteriophage PNM (a member of the *Podoviridae* family). **(B)** Member of the *Myoviridae* family with contracted tail. **(C)** Member of the *Myoviridae* family with normal tail. **(D)** Member of the *Myoviridae* family. **(E)** Three PNM bacteriophages attached to *P. aeruginosa* (two of them have injected their DNA, the heads are empty). **(F)** *P. aeruginosa* under the attack of bacteriophages. **(G)** *P. aeruginosa* under the attack of bacteriophage 14/1, a member of the *Myoviridae* family (the bacteriophages breached the cell wall, causing leakage of cell content and subsequent death of the bacterium). **(H)** Cluster of newly formed 14/1 bacteriophages. **(I)** *Staphylococcus aureus* intravenous *Staphylococcus* phage bacteriophages (members of the *Myoviridae* family) attached to a dividing *S. aureus* bacterium.
The electronmicrographs were made by Dr Jan Mast of the Veterinary Agrochemical Research Center in Brussels.

Europe. The current European Medicinal Products for Human Use regulation was established with classical drug products in mind. Today, this procedural pathway works as a 'resistance mechanism' for an eventual breakthrough of phage therapy in Europe. Indeed, in the actual regulatory setting, it is hard, if not impossible, to launch the clinical studies that are required to generate the data demonstrating safety and efficacy of phage therapy.

Basic problem

A fundamental aspect in the discussion surrounding the clinical application of bacteriophages is the biological status and definition of viruses in general, and, more specifically, bacteriophages.

A virus is a natural biological entity, a molecular, genetic, replicative parasite. But, is it living? From one perspective, we cannot say viruses are living organisms since they are acellular and have no metabolism, however, they can self-replicate and evolve, two typical aspects of life. Viruses, however, only exhibit these life characteristics when they are immersed in a specific cellular environment. Viruses are the most abundant biological lifelike entities on earth according to the latest scientific insights, and as such are major players in the evolution of living systems. Furthermore, they seem to have played an essential role in the emergence of cellular (organismal) life [7–8]. Today, some scientists put forward the idea of an extended definition of life and a revision of the tree of life. This provocative idea was recently discussed and exposed by Peter Ward in his book *Life As We Do Not Know It* [9].

Regulatory conundrum

European regulation defines a medicinal product as 'any substance presented for treating or preventing disease in human beings' [10–11]. A 'substance' is defined as any matter, irrespective of origin. As such, even microorganisms or whole animals fall under this regulation. Today leeches and fly larvae, for example, are classified as medicinal products.

According to this definition, therapeutically used bacteriophages are medicinal products. In today's practice, however, it is impossible to document bacteriophages as if they were medicinal products.

The Gene Therapy section of the *Advanced Therapy Medicinal Product Regulation* describes, amongst others, the use of genetically modified viruses as vectors targeting eukaryotic cells for gene therapy. However, this does not apply to bacteriophages since they are not genetically modified and have no affinity for eukaryotic cells. No bacteriophage DNA was found in our genetic makeup, in contrast to the high number of retroviral remnants present in our core genome. Some bacteriophage-related polymerase gene sequences were identified in mitochondrial DNA. Mitochondria originated from ancestral Rickettsia-like bacteria that began a symbiotic relationship with prototype eukaryotic cells (similar to chloroplasts in plants). This ancient process dates back from the endosymbiotic era at the time of the evolutionary split between pro- and eukaryotes. The phage DNA was most probably introduced during the bacterial phase of the mitochondrion.

Starting a clinical trial, based on the submission of an incomplete file in the official medicinal product pathway (national notification, Eudract number, production license etc.) could put the investigators and the study at risk. In fact, we are faced with a vicious circle in which we are prevented from beginning the clinical studies necessary to achieve an adequate regulation.

Probiotics

Bacteriophages are extremely abundant, ubiquitous and present in environments as diverse as sea water, drinking water, activated sludge, food and cosmetics, and inhabit our bodies in at least as large numbers as bacteria. Unknowingly we constantly consume bacteriophages with our drinking water and food (e.g., yogurt, cheese, salami etc.). An equilibrated food intake is the basis for health.

Probiotics such as active-bifidus containing yoghurts, also available on the European market, are claiming to have a positive effect on our health through the restoration of the intestinal flora. This means, in fact, that there is a commercial claim that these yoghurts generate positive therapeutic effects. However, at the regulatory level, the therapeutic effects for this type of product are not claimed, since otherwise such yoghurts would be considered medicinal products according to European regulation.

Fermented-milk drinks containing living *Lactobacillus* spp. such as *Lactobacillus casei shirota* or *Lactobacillus casei immunitas*, but also their respective bacteriophages, are also present on the European market. Based on the Japanese Foods for Specified Health Use (FOSHU) Regulation, the following therapeutic effects are claimed: regulation of the gastrointestinal condition, reduction of harmful bacteria and suitable for therapeutic use against acute diarrhea [12–13]. These products did not receive the medicinal product status either.

In The Netherlands, these milk drinks claim to improve bowel habits in subjects who are susceptible to constipation and to support a well-balanced gut microbiome through an increase in the number of *Lactobacilli*. These claims were validated by a committee of independent scientists on specific request of The Netherlands Nutrition Center and a major milk-drink manufacturer. Despite these valorized claims, the product was not classified as a medicinal product [14,101].

Apparently, for food products, therapeutic claims do not have to be backed up by clinical (safety) trials or, in fact, by any study whatsoever.

'Generally recognized as safe' product regulation

Some companies decided to penetrate the food market in order to accustom the public and the regulatory authorities to bacteriophages, which should facilitate future clinical trials whilst already generating certain revenues. The application of phages in the agrobio industry is also a good alternative to antibiotics and represents a considerable market.

Therefore, in the USA, the FDA recently (April 2006) approved the use of bacteriophage-cocktails against *Listeria monocytogenes* in ready-to-eat meat and poultry. The FDA classified these bacteriophages under the generally recognized as safe (GRAS) product regulation [15–16].

Declaration of Helsinki

In Poland, a recent member of the European Union, bacteriophages are already therapeutically used. The Polish Academy of Sciences and L. Hirszfeld Institute of Immunology and Experimental Therapy offers phage-therapy to treat patients infected with drug-resistant bacteria [102].

The regulatory basis for this therapeutic use of bacteriophages on patients is the Declaration of Helsinki. Paragraph 32 of this Declaration states:

> *"In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published".*

The other relevant guidelines of this Declaration should be followed.

In June 2005, the Ethical Committee of the Medical Academy in Wroclaw authorized a study named *Experimental Phage Therapy in Antibiotic-Resistant Bacterial Infection, Including MRSA Infection*. Neither the European Medicinal Product Regulation nor the Polish National translation of this regulation was applied and Europe did not oppose to this.

Brussels Burn Wound Center

The Burn Wound Center of the Queen Astrid Military Hospital cares for 1350 patients (10,000 consultations) yearly and, as in most hospitals, clinicians are increasingly confronted with multidrug resistant (MDR) bacteria causing virtually untreatable infections [17].

In our function as biomedical researchers, independent from the pharmaceutical industry, we suggested to our clinicians the application of bacteriophages as an alternative for antibiotics in the treatment of MDR bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), MDR *Pseudomonas aeruginosa* and MDR *Acinetobacter baumannii*.

An immediate application of phages covered by the Declaration of Helsinki was out of the question in our center. We wanted to set up a proof-of-concept clinical trial in a well-specified burn-wound patient population with well-defined bacteriophages targeted against specific bacterial species/strains. Furthermore, the Declaration of Helsinki is only applicable when therapeutic methods do not exist or have been ineffective. When applied correctly, the Declaration of Helsinki can only justify the application of bacteriophages in patients with untreatable infections caused by MDR bacteria. This is only the case for a few patients/year in our center. As a consequence we feel this is not a durable solution for phage therapy.

Alternatives

Several alternatives were taken into consideration. First, we considered asking our hospital pharmacist to prepare the phage cocktails as if they were magistral preparations. Magistral preparations, however, are normally prepared using EU-registered product components, of which phages are not.

Second, we considered using bacteriophages under the compassionate use umbrella. This was abandoned because this rule only applies to non-registered drugs that are already in a clinical-study phase and have proven a certain potential.

We also considered going for the 'orphan-drugs' approach. The use of phages on a limited population of patients (e.g., third-degree burn wound or cystic fibrosis patients) could justify this option. But, this does not solve the documentation problem either, since in this setting the bacteriophage cocktail remains to be documented as a medicinal product.

Finally, we decided to try to conduct a limited clinical trial with the authorization of, and under the supervision of, a Belgian leading Medical

Ethical Committee that was provided with the standard required detailed documentation (product information, study protocol, 'no fault' insurance, informed consent etc.). The submitted clinical trial consists of the application of a characterized (fingerprint and electron microscopy), targeted (against the bacteria in the Burn Wound Center), safe (sterility and apyrogenicity certified by accredited organizations), truly lytic and noncytotoxic bacteriophage cocktail in thermally injured patients with specific infections caused by MRSA and/or MDR *P. aeruginosa* strains. All the questions of the Medical Ethical Committee were answered satisfactorily. On the 20th June 2007, we obtained the approval of a Belgian leading Medical Ethical Committee to conduct a limited clinical trial. A well-defined bacteriophage cocktail targeted against the *P. aeruginosa* and *S. aureus* strains present in our Burn Wound Center will be applied on infected or colonized burn wounds in 20 patients.

In the evaluation process of the study we were confronted with the false perception of viruses as 'enemies of life', an observation that was previously expressed by Luis Villareal [18]. This, amongst others, resulted in a tenfold increase of the study insurance fee and an unwarranted request to notify the National Bio-safety Council, which technically only rules on the release of genetically modified organisms and pathogenic microorganisms in the environment. This was a false perception, since phage therapy has been proven safe through the massive application of lytic bacteriophages in humans, mostly in former USSR states [19–21] and in animal studies [22–26] and safety studies in healthy humans [27] in the Western world. To our knowledge, no specific or lethal product-related adverse events were reported.

Ultimately, we would like to support a long-term solution, such as the creation of a specific section for phage therapy under the Advanced Therapy Medicinal Product Regulation included in the European Directive 2003/63/EC, which was translated into Belgian Law by the Royal Decree of March 4, 2004 and presently includes Gene Therapy and Somatic Cell Therapy. A third section, Tissue Engineered Products, is in preparation. We propose the creation of a fourth section named Bacteriophage Therapy Preparations, which could be divided into two distinct sections: 'tailor made use in a hospital environment' versus 'industrially produced for market placement'. This specific regulation should allow nonprofit organizations such as hospitals to apply in-house phage therapy without hampering pharmaceutical companies in the development of commercial phage preparations.

Conclusion

The current European regulatory setting only allows for eventual sporadic clinical trials under the responsibility and supervision of the Medical Ethical Committees and/or border-line applications under the umbrella of the Declaration of Helsinki. There is a clear need for an adapted European regulation tailored to phage therapy, allowing the justified and broad therapeutic use of bacteriophages as an alternative or complement to antibiotics. The absence of a rational regulation will leave a sterile discussion between phage skeptics, who claim scientific evidence, and phage enthusiasts, willing to test phage therapy objectively but prevented to do so by high administrative and regulatory hurdles.

Future perspective

The concept of selecting a mixture of various phages would enable one to effectively target various bacteria. This can be applied to hard-to-treat infected wounds or, with an appropriate preparation, even against some systemic infections. Furthermore, as phages can be lyophilized and stored for quite a long time, ready-to-use cocktails could be used to fight outbreaks or other public health emergencies, even in the context of 'bio-defense'. But, we are not there…yet. In the future, regulation should be adapted in order to give phage therapy a chance. We cannot leave any opportunity to solve the antibiotic-resistance problem unexplored. From an economical point of view, phage therapy could be a hidden fortune.

Acknowledgements

We thank Dr Jean Pirson, head of the Burn Wound Center, Dr Kenneth Coenye, surgeon, and Dr Patrick Soentjens, infectious disease specialist, for their immediate interest and openness concerning the use of bacteriophages as an alternative for antibiotics.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Phage therapy as an alternative for antibiotics

- Treatment of infectious diseases with antibiotics is becoming increasingly challenging.
- Very few new antimicrobials are in the pipeline of the pharmaceutical industry.
- One of the potential alternatives for antibiotics is phage therapy.

Obstacles

- False perception of viruses as 'enemies of life'.
- No specific frame for phage therapy in the current Medicinal Product Regulation.

Solutions

- Short-term borderline solutions:
  - Declaration of Helsinki
  - Medical Ethical Committee approval
- Long-term solution:
  - Creation of a specific section for phage therapy under the Advanced Therapy Medicinal Product Regulation.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Levy SB, Marshal B: Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10, S122–S129 (2004).
2. Martinez JL, Baquero F: Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity and antibiotic resistance. *Clin. Microbiol. Rev.* 15, 647–679 (2002).

•• **Well-written paper with a global scientific as well as clinical viewpoint.**

3. Baquero F, Coque TM, Canton R: Antibiotics, complexity and evolution. *ASM News* 69, 547–552 (2003).

•• **Clearly written article bringing insights from the fundamental systematics and evolutionary biology into the antibiotic resistance problem for a broad audience.**

4. Pirnay JP, De Vos D, Zizi M, Heyman P: No easy way to exterminate 'superbugs' at the dawn of the third millennium. *Expert Rev. Anti Infect. Ther.* 1(4), 523–525 (2003).
5. Kutter E, Sulakvelidze A: In: *Bacteriophages Biology and Applications.* Kutter E, Sulakvelidze A (Eds). CRC Press Boca Raton, FL, USA (2005).

•• **Well-written and documented textbook, including various historical and actual aspects in the field.**

6. Gorski A, Borowski J, Miedzybrodzki R, Weber-dabrowska B. In: *Bacteriophage: Genetic and Molecular biology*. Mcgrath S, van Sinceren D (Eds). Horizon Scientific Press, UK 125–157 (2007).

•• **Recent chapter of a new textbook on various aspects, including the regulation of phage therapy.**

7. Bamford DH: Do viruses form lineages across different domains of life? *Res. Microbiol.* 154, 231–236 (2003).

•• **Very interesting paper discussing the viral world based on recent new sequence data and fundamental aspects, such as the idea that there is supporting evidence for a redefinition of 'life' from a cell-centered description toward a viro-centered one.**

8. Forterre P: The origin of viruses and their possible roles in major evolutionary transitions. *Virus Res.* 117, 5–16 (2006).

•• **Thoughtfully written paper concentrating on the different origins of viruses and their role in the emergence and evolution of life, and their role in the major evolutionary transitions, such as the pro/eukaryotic split.**

9. Ward P: In: *Life As We Do Not Know It.* Ward P (Ed.). Viking Penguin, NY, USA (2005).

• **Well written book for a nonvirologist or nonfundamental biologist audience, summarizing the latest ideas of 'what life is and especially what life could be', including the status of viruses.**

10. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal of the European Communities (translated into Belgian Law by Royal Decree of 04.03.2004), published in the Belgian Official Journal on 10.03.2004.
11. Commission Directive 2003/63/EC of 25 June 2003 amending Directive 2001/83/EC of the European Parliament and the council of the Community code relating to medicinal products for human use. Official Journal of the European Union (translated into Belgian Law by Royal Decree of 04.03.2004), published in the Belgian Official Journal on 10.03.2004.

•• **Basic text concerned with medicinal product regulation. It is in this text that we would like to see the creation of a paragraph under part IV regulating the 'advanced therapy issues'.**

12. Sanders ME, Huis in't Veld J: Bringing a probiotic-containing functional food to the market: microbiological, product, regulatory and labeling issues. *Antonie van Leeuwenhoek* 76, 293–315 (1999).

• **Interesting article on the basic probiotic issues.**

13. Berman SH, Eichelsdoerfer P, Yim D, Elmer GW, Wenner CA: Daily ingestion of a nutritional probiotic supplement enhances innate immune function in healthy adults. *Nutr. Res.* 26, 454–459 (2006).
14. Agarwal KN, Bhasin SK: Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations Actimel and Indian Dahi. *Eur. J. Clin. Nutr.* 56(S4), 556–559 (2002).
15. FDA 21 CFR Part 172: Food Additives Permitted for Direct Addition to Food for Human Consumption; Bacteriophage Preparation. Federal Register, Rules and Regulations No. 160. 71, 47729–47732 (2006).
16. Burdock GA, Carabin IG: Generally recognized as safe (GRAS): history and description. *Toxicol. Lett.* 150, 3–18 (2004).
17. Pirnay JP, De Vos D, Cochez C *et al.*: Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. *J. Clin. Microbiol.* 41(3), 1192–1202 (2003).

18. Villareal LP: Overall issues of virus and host evolution. In: *Viruses and the Evolution of Life.* Villareal LP (Ed.). ASM Press, WA, USA 1–28 (2005).
19. Dabrowska K, Switala-Jelen K, Opolski A, Weber-Dabrowska B, Gorski A: Bacteriophage penetration in vertebrates. *J. Appl. Microbiol.* 98, 7–13 (2003).

•• **Very good, complete review with emphasis on vertebrate host and phage interactions, including potential lethal issues.**

20. Brüssow H: Phage therapy: The *Escherichia coli* experience. *Microbiology* 151, 2133–2140 (2005).

•• **Very good general review on phage therapy, including historical and *in vivo* studies on man and animals.**

21. Matsuzaki S, Rasbel M, Uchiyama J *et al.*: Bacteriophage therapy: a revitalized therapy against bacterial infections diseases. *J. Infect. Chemother.* 11, 211–219 (2006).
22. Biswas B, Adhya S, Washart P *et al.*: Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect. Immun.* 70, 204–210 (2002).
23. Chibani-Chennoufi S, Sidoti J, Brüttin A, Kutter E, Sarket S, Brüssow H: *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob. Agents Chemother.* 48, 2558–2569 (2004).
24. Wills QF, Kerrigan C, Soothill JS: Experimental bacteriophage protection against *Staphylococcus aureus* abscess in a rabbit model. *Antimicrob. Agents Chemother.* 49, 1220–1221 (2005).
25. Marza JA, Soothill JS, Boydell P *et al.*: Muliplication of therapeutically administred bacteriophages in *Pseudomona aeruginosa* infected patients. *Burns* 32, 644–646 (2006).
26. McVay CS, Velasquez M, Fralick JA: Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. *Antimicrob. Agents Chemother.* 51, 1934–1938 (2007).
27. Brüttin A and Brüssow H: Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.* 49, 2874–2878 (2005).

Websites

101. The Netherlands Nutrition Center www.voedingscentrum.nl/NR/rdonlyres/4E6011C9–7FED-47B4-A31A-EDA304693947/0/ASSESSMENTREPORT061110.pdf
102. Polish Academy of Sciences, Institute of Immunology and Experimental Therapy. Therapeutic use of bacteriophages in bacterial infections www.aite.wroclaw.pl/phages/phages.html

Affiliations

- *Gilbert Verbeken*
  Hospital Center of the Base – Queen Astrid, Laboratory for Molecular & Cellular Technology, Burn Unit, Bruynstraat 1, B-1120 Neder-over-Heembeek, Belgium
  Tel.: +32 2264 4856;
  Fax: +32 2264 4907;
  gilbert.verbeken@mil.be
- *Daniel De Vos*
  Hospital Center of the Base – Queen Astrid, Laboratory for Molecular & Cellular Technology, Burn Unit, Bruynstraat 1, B-1120 Neder-over-Heembeek, Belgium
  Tel.: +32 2264 4856;
  Fax: +32 2264 4907;
  daniel.devos2@mil.be
- *Mario Vaneechoutte*
  Ghent University Hospital, Laboratory of Bacteriology & Virology, De Pintelaan 185, B-9000 Ghent, Belgium
  Tel.: +32 9240 3692;
  Fax: +32 9240 3659;
  mario.vaneechoutte@ugent.be
- *Maya Merabishvili*
  Eliava Institute of Bacteriophage, Microbiology & Virology, 3 Gotua Street, 380060 Tbilisi, Georgia
  Tel.: +32 9240 3692;
  Fax: +32 9240 3659;
  m_merabish.ibmv@caucasus.net
- *Martin Zizi*
  Brussels University Hospital, Department of Physiology, Laarbeeklaan 101, B-1090 Brussel, Belgium
  Tel.: +32 2477 4434;
  Fax: +32 2477 4000;
  martin.zizi@vub.ac.be
- *Jean-Paul Pirnay*
  Hospital Center of the Base – Queen Astrid, Laboratory for Molecular & Cellular Technology, Burn Unit, Bruynstraat 1, B-1120 Neder-over-Heembeek, Belgium
  Tel.: +32 2264 4844;
  Fax: +32 2264 4907;
  jean-paul.pirnay@mil.be

2.2 Development of a selection and production scheme for bacteriophages used in a clinical setting (Study 2)

2.2.1 Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials

M. Merabishvili, J.P. Pirnay, G. Verbeken, N. Chanishvili, M. Tediashvili, N. Lashkhi , T. Glonti, V. Krylov, J. Mast, L. Van Parys, R. Lavigne, G. Volckaert, W. Mattheus, G. Verween, P. De Corte, T. Rose, S. Jennes, M. Zizi, D. De Vos, M. Vaneechoutte

Plos ONE. 2009; 4(3):e4944

International scientific journal, peer-reviewed

2.2.1 Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials

M. Merabishvili, J.P. Pirnay, G. Verbeken, N. Chanishvili, M. Tediashvili, N. Lashkhi , T. Glonti, V. Krylov, J. Mast, L. Van Parys, R. Lavigne, G. Volckaert, W. Mattheus, G. Verween, P. De Corte, T. Rose, S. Jennes, M. Zizi, D. De Vos, M. Vaneechoutte

Plos ONE. 2009; 4(3):e4944

International scientific journal, peer-reviewed

OPEN ACCESS Freely available online

PLoS one

Quality-Controlled Small-Scale Production of a Well-Defined Bacteriophage Cocktail for Use in Human Clinical Trials

Maya Merabishvili[1,2,8], Jean-Paul Pirnay[2]*, Gilbert Verbeken[2], Nina Chanishvili[1], Marina Tediashvili[1], Nino Lashkhi[1], Thea Glonti[1], Victor Krylov[3], Jan Mast[4], Luc Van Parys[5], Rob Lavigne[6], Guido Volckaert[6], Wesley Mattheus[6], Gunther Verween[2], Peter De Corte[2], Thomas Rose[2], Serge Jennes[2], Martin Zizi[5,7], Daniel De Vos[2], Mario Vaneechoutte[8]

1 Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV), Tbilisi, Georgia, **2** Laboratory for Molecular and Cellular Technology (LabMCT), Burn Centre, Queen Astrid Military Hospital, Bruynstraat, Neder-over-Heembeek, Brussels, Belgium, **3** Laboratory of Bacteriophage Genetics, State Institute for Genetics and Selection of Industrial Microorganisms (SIGSIM), Moscow, Russia, **4** Unit Electron Microscopy, Veterinary and Agricultural Research Centre (VAR), Ukkel, Brussels, Belgium, **5** Section Health of the Division Well-Being (Belgian Defence Staff), Queen Astrid Military Hospital, Neder-over-Heembeek, Brussels, Belgium, **6** Laboratory of Gene Technology (LoGT), Katholieke Universiteit Leuven, Leuven, Belgium, **7** Department of Physiology (FYSP), Vrije Universiteit Brussel, Jette Brussels, Belgium, **8** Laboratory of Bacteriology Research (LBR), Ghent University Hospital, Ghent, Belgium

Abstract

We describe the small-scale, laboratory-based, production and quality control of a cocktail, consisting of exclusively lytic bacteriophages, designed for the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections in burn wound patients. Based on succesive selection rounds three bacteriophages were retained from an initial pool of 82 *P. aeruginosa* and 8 *S. aureus* bacteriophages, specific for prevalent *P. aeruginosa* and *S. aureus* strains in the Burn Centre of the Queen Astrid Military Hospital in Brussels, Belgium. This cocktail, consisting of *P. aeruginosa* phages 14/1 (*Myoviridae*) and PNM (*Podoviridae*) and *S. aureus* phage ISP (*Myoviridae*) was produced and purified of endotoxin. Quality control included Stability (shelf life), determination of pyrogenicity, sterility and cytotoxicity, confirmation of the absence of temperate bacteriophages and transmission electron microscopy-based confirmation of the presence of the expected virion morphologic particles as well as of their specific interaction with the target bacteria. Bacteriophage genome and proteome analysis confirmed the lytic nature of the bacteriophages, the absence of toxin-coding genes and showed that the selected phages 14/1, PNM and ISP are close relatives of respectively F8, ϕKMV and phage G1. The bacteriophage cocktail is currently being evaluated in a pilot clinical study cleared by a leading Medical Ethical Committee.

Citation: Merabishvili M, Pirnay J-P, Verbeken G, Chanishvili N, Tediashvili M, et al. (2009) Quality-Controlled Small-Scale Production of a Well-Defined Bacteriophage Cocktail for Use in Human Clinical Trials. PLoS ONE 4(3): e4944. doi:10.1371/journal.pone.0004944

Editor: David M. Ojcius, University of California Merced, United States of America

Received November 26, 2008; **Accepted** February 13, 2009; **Published** March 20, 2009

Funding: MM, JPP, GiV, LVP, GuV, PDC, TR, SJ, MZ and DDV were financially supported by the Belgian Ministry of Defence. This work was partially supported by the research council of the K.U.Leuven (OT/05/47) and grant WB15 of the Royal High Institute for Defence (RHID). Selection and initial characterisation of the phages was financed by INTAS project 6610. Laboratories 1, 2, 3 and 6 are part of the research community 'PhageBiotics', supported by the FWO Vlaanderen. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: jean-paul.pirnay@mil.be

Introduction

In burn wound care, bacterial infection remains a major therapeutic problem and renders large numbers of thermal injuries virtually untreatable. Whereas *Staphylococcus aureus* remains a common cause of early burn wound infection, *Pseudomonas aeruginosa* is known as the most common and lethal infectious agent in burn centres, essentially due to its intrinsic and acquired resistance to antibiotics [1–3].

To improve burn wound patient care, research at the Laboratory for Molecular and Cellular Technology (LabMCT) of the Burn Centre of the Queen Astrid Military Hospital in Neder-over-Heembeek (Brussels), Belgium is investigating alternatives for the treatment of infections with multidrug resistant (MDR) infectious agents, like (bacterio)phage therapy. The use of bacteriophages against bacterial pathogens was first proposed by d'Hérelle in 1917 [4] and has a long and convoluted history. Currently, phage therapy is the subject of renewed interest, as a consequence of the continuing increase in antibiotic resistance worldwide [5], illustrated by the growing number of scientific papers and text books [6–12].

However, major obstacles for the clinical application of bacteriophages are the perception of viruses as 'enemies of life' [13], the lack of a specific frame for phage therapy in the current Medicinal Product Regulation [14] and the absence of well-defined and safe bacteriophage preparations.

To evaluate the safety and efficacy of bacteriophages in the treatment of burn wound infections in a controlled clinical trial, we prepared a highly purified and fully defined bacteriophage cocktail (BFC-1), active against the *P. aeruginosa* and the *S. aureus* strains actually circulating in the Burn Centre of the Queen Astrid Military.

To our knowledge the present paper describes for the first time, in detail - from the initial bacteriophage isolation to the final composition - a laboratory-based production of a well-defined bacteriophage cocktail.

Methods

A flow chart of the entire BFC-1 production process and quality control tests is depicted in **Figure 1**.

Titration of bacteriophage suspensions using the agar overlay method

The bacteriophage titre was determined by assaying decinormal serial dilutions (log(0) to log(−12)) of the bacteriophage suspensions with the agar overlay method [27,28]. One ml of each dilution was mixed with 2.5 ml molten (45°C) Luria Bertani (LB) (Becton Dickinson, Erembodegem, Belgium), containing 0.7% top agar (Bacto agar, Becton Dickinson), and a suspension of bacteriophage sensitive bacteria (end concentration of 10^8 cfu/

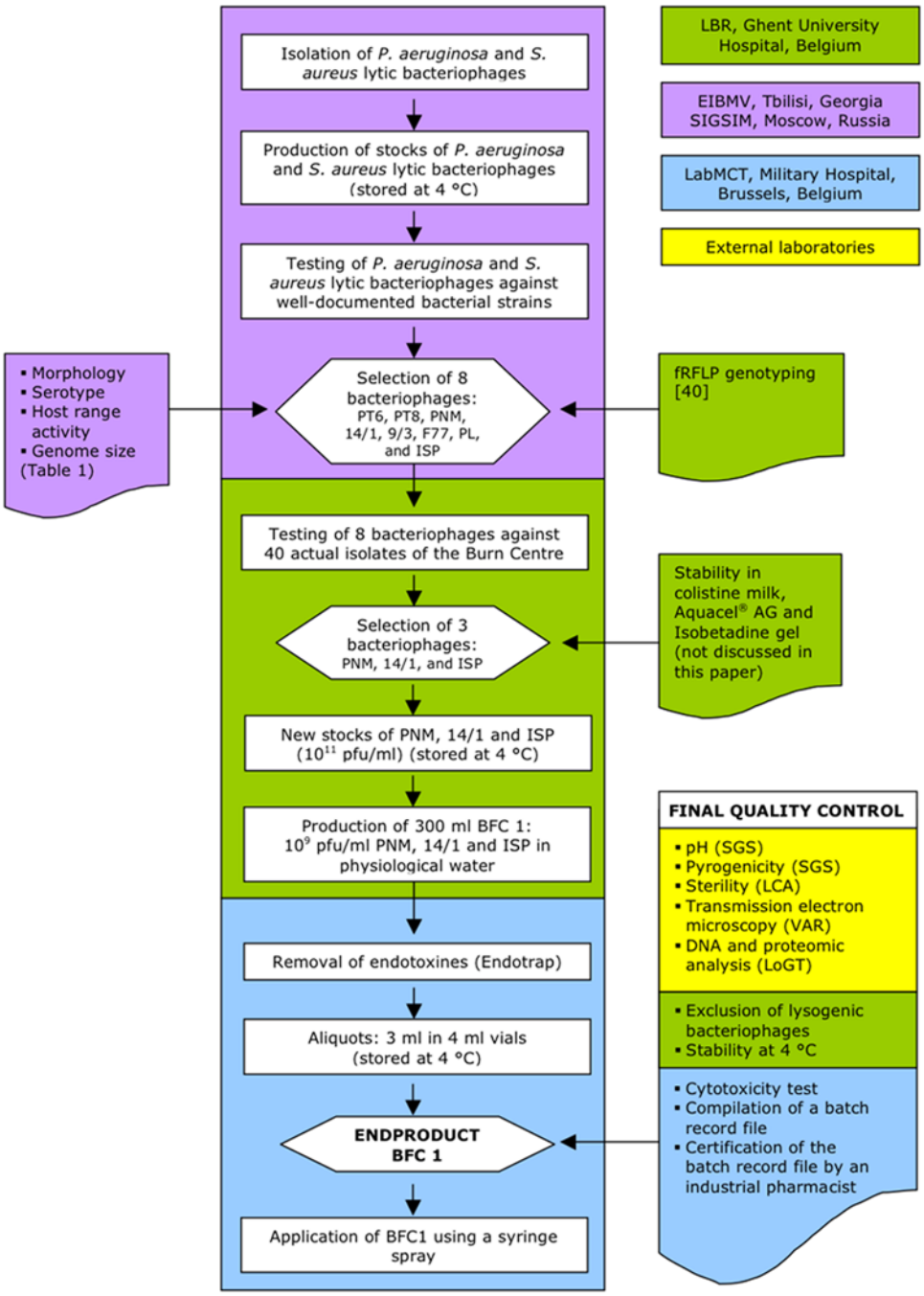


Figure 1. Flow chart of the BFC-1 production and final quality control.
doi:10.1371/journal.pone.0004944.g001

ml) in sterile 14 ml tubes (Falcon, Becton Dickinson). This mixture was plated in triplicate onto 90 mm diameter Petri dishes (Plastiques Gosselin, Menen, Belgium) filled with a bottom layer of 1.5% LB agar and incubated for 18–24 h at 37°C. To estimate the original bacteriophage concentration, plates with one to 100 distinguishable homogenous plaques were counted depending on the phage plaque size. The mean was then calculated for the triplicate plates.

Initial isolation, separation and purification of *P. aeruginosa* and *S. aureus* lytic bacteriophages

The bacteriophage sensitive strains used during the production and quality control of BFC-1 are *P. aeruginosa* strain '573', were isolated at the Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV) in the 1970s from bone marrow interstitial fluid, and *S. aureus* strain '13 S44 S', isolated at the Brussels Burn Centre in 2006 from a burn wound. Initially, *S. aureus* strain Wood 60 (EIBMV collection) was used for propagation of phage ISP, but for the production of this cocktail the phage was propagated on *S. aureus* 13 S44 S. The absence of temperate phages from the host strains was tested as described in a separate section of this paper.

For bacteriophage isolation from natural samples such as sewage and river water, one millilitre of 10×concentrated LB Broth (Becton Dickinson), 1 ml 'host bacteria' suspension, containing 10^8 cfu in LB broth and 9 ml sewage or river water were mixed in a 14 ml sterile tube. This tube was incubated at 37°C for 1.5–2 h. Subsequently, 200 µl of chloroform (Sigma-Aldrich, Bornem, Belgium) was added and the tube was further incubated at 4°C for 1 h. The lysate was aspirated with a sterile 5 ml syringe and passed through a 0.45 µm membrane filter (Minisart, Sartorius, Vilvoorde, Belgium). Bacteriophages were titrated using the agar overlay method, as described above. All plaques with different morphology were touched with a sterile pipette tip, inoculated into 2 ml of sterile LB broth in 14 ml sterile tubes and incubated at 37°C for 2 h. Subsequently, 50 µl of chloroform was added and the tube(s) were incubated at 4°C for 1 h. For each tube, a dilution series (log(0)−log(−4)) was made in sterile 14 ml tubes filled with LB broth. Each dilution was titrated using the agar overlay method. Plates showing 1–10 plaques were analysed in detail. Again, all plaques with different morphology were touched with a sterile pipette tip, inoculated into 2 ml of sterile LB broth in 14 ml sterile tubes and incubated at 37°C for 2 h. This complete cycle was repeated until one plaque morphotype was obtained (homogeneous plaques).

In the case of bacteriophage ISP, which was isolated in the 1920s, porcelain, rather than membrane filters were employed.

Production of bacteriophage stocks

Bacteriophage stocks were prepared using the double-agar overlay method with minor modifications. One millilitre of lysate (see above) containing 10^3–10^5 plaque forming units (pfu) of bacteriophages was mixed with 2.5 ml molten (45°C) Select Alternative Protein Source (APS) LB (Becton Dickinson, Erembodegem, Belgium) top agar (0.7%) and a bacteriophage sensitive bacterial suspension (end concentration of 10^8 cfu/ml) in a sterile 14 ml tube. This mixture was plated onto ten 90 mm diameter Petri dishes filled with a bottom layer of 1.5% APS LB agar and incubated at 37°C for 16–18 h. Subsequently, 200 µl of chloroform was added to the lids of the Petri dishes and further incubated at 4°C for 1 h. The top agar layer was scraped off using a sterile Drigalski spatula (L-shaped rod) and transferred to a sterile 14 ml tube. The mixture was centrifuged for 20 min at 6 000 g. The supernatant was aspirated using a sterile 10 ml syringe (BD Plastipak, Becton Dickinson) with a 30 G sterile needle (BD microlance 3, Becton Dickinson) and passed through a 0.45 µm membrane filter.

Selection of therapeutic bacteriophages

Large 24.5 cm square Petri dishes (Nunc, Wiesbaden, Germany) with 2% LB agar were inoculated with the target bacteria (10^8 cfu/ml LB broth). Each target bacterium was applied in one horizontal strip. As a consequence, the dish contained multiple parallel inoculation strips. Each strip was air-dried and spotted with 5 µl of 10^7 pfu/ml of each of the bacteriophage suspensions under consideration. The dish was incubated for 16–18 h at 37°C. The obtained lysis zones were evaluated and scored as cl (confluent lysis), ol (opaque lysis), scl (semi-confluent lysis), sp (several plaques) and – (negative reaction).

Composition and endotoxin purification of the bacteriophage cocktail

Each of the three bacteriophage stock suspensions (PNM, 14/1 and ISP) of the final cocktail was diluted into 100 ml of a sterile 0.9% NaCl solution (B. Braun, Diegem, Belgium) to a final concentration of 3.10^9 pfu/ml. Subsequently, the three bacteriophage suspensions were mixed in a sterile 500 ml PETG Nalgene® bottle (Nalge Europe, Neerijse, Belgium) to obtain a 300 ml volume of the bacteriophage cocktail (named BFC-1), which contained each bacteriophage at a concentration of 10^9 pfu/ml. BFC-1 was subsequently purified from endotoxins using a commercially available kit (EndoTrap® Blue, Cambrex BioScience, Verviers, Belgium), according to the instructions of the manufacturer. One column was utilised per 50 ml of BFC-1. Endotoxin purified BFC-1 was collected into a sterile 500 ml PETG Nalgene® bottle and aliquoted into 3 ml doses in sterile 4 ml vials (Brand, Wërtheim, Germany). The final titre of each phage was approximately 1.10^9 pfu/ml.

Final quality control

pH. The pH of BFC-1 was determined by an accredited laboratory (SGS Lab Simon AS, Brussels, Belgium) in accordance to the European Pharmacopoeia standards (EP6).

Pyrogenicity

Pyrogenicity was tested by an accredited laboratory (SGS Lab Simon SA) in accordance with the European Pharmacopoeia standards (EP6). A sample of 1.2 ml BFC-1 was intravenously injected in three rabbits. The calculation of the rabbit injection volume in order to achieve the maximum safety level was done according to the following equation: rabbit injection volume = human injection volume x safety factor x rabbit weight/70, with human injection volume = 3 ml - whereby 3 ml is the maximal volume topically applied in the clinical trial and immediate and complete resorption is assumed, to achieve maximal safety; with safety factor = 8 (i.e. maximum safety level) and with rabbit weight = 3.5 kg.

Sterility

Ten percent of the BFC-1 production was tested for sterility by an accredited laboratory (Laboratoire de Contrôle et d'Analyses, Brussels, Belgium) using the membrane filtration method followed by two weeks of incubation at 37°C, in accordance to the European Pharmacopoeia (EP6).

Transmission electron microscopy

Bacteriophage particles, with and without target bacteria were analysed by transmission electron microscopy as described by Imberechts et al. [29]. Briefly, suspensions were brought on

carbon and pioloform-coated grids (Agar Scientific, Stansted, UK), washed with water and negatively stained with 2% uranyl acetate (Agar Scientific) in water and analyzed using a Technai Spirit transmission electron microscope (FEI, Eindhoven, The Netherlands) operating at 120 kV. Micrographs were recorded using a bottom-mounted digital camera (Eagle, 4X4K, FEI).

Exclusion of temperate bacteriophages from the host strains

To confirm the absence of temperate bacteriophages, originating from the bacterial hosts used to grow the three lytic bacteriophages, a standard technique for bacteriophage induction using the DNA-damaging antimicrobial agent mitomycin C was carried out, as described by Miller [30].

The host strains used in BFC-1 production (*P. aeruginosa* strain 573 and *S. aureus* strain 13 S44 S, as well as the *P. aeruginosa* reference strain 'PAO1') were grown in APS LB broth at 37°C until the early exponential growth phase. Bacterial cultures were aliquoted in 1 ml volumes in sterile eppendorf tubes, covered with aluminium foil thus protecting the bacteria from photoreactivation of drug-induced DNA damage. Mitomycin C (Sigma-Aldrich) was added to final concentrations of 1 or 5 μg/ml [30]. A control tube without mitomycin C was added to evaluate the presence of 'non-drug-induced' bacteriophages. The tubes were incubated for 3 h at 37°C. Subsequently, twenty μl of chloroform was added to the control tubes to lyse the bacteria. The lysates were centrifuged in order to separate the intact bacterial cells from the supernatant. The final titre of bacteriophages in the supernatant was determined using the double agar overlay method, as described above.

Genome sequencing and proteomic analysis

DNA from bacteriophages was isolated using a commercially available kit (Lambda Mini Kit, Qiagen, Hilden, Germany). Purified DNA from bacteriophages 14/1, PNM and ISP was sonicated for 1 s at 20% intensity using a Sonics Vibracell and separated on a 1% agarose gel (Eurogentec, Seraing, Belgium). Fragments between 1000 and 2000 bp were excised from the gel (Qiagen, Venlo, The Netherlands), end repaired using Klenow - T4 polymerase mixture (Fermentas, St.-Leon-Rot, Germany) and phosphorylated using T4 polynucleotide kinase (Roche, Vilvoorde, Belgium). Subsequently, fragments were ligated using T4 ligase (Fermentas) into *Sma*I-linearised pUC19 plasmids and transformed to *Escherichia coli* XL1 blue MRF. This resulted in over 10 000 positive clones after blue white screening for each bacteriophage, of which more than 90% contained inserts between 1 and 2 kb. Plasmids from individual clones were isolated and sequenced using the standard M13f vector primer. After standard ethanol precipitation, samples were separated and analyzed on an ABI 3130 capillary sequencing device (Applied Biosystems, Lennik, Belgium). To complete the genomes (sequenced from each strand for each position), standard primer walking was used. Sequence assembly into contigs was performed using Sequencher 4.1 software (Gene Codes Corporation, Ann Arbor, USA). ORF predictions were made using comparative genomics approaches (tBLASTx), searching for conserved gene products between closely related phages (14-1 vs F8, PNM vs phiKMV and ISP vs G1). To scan the genomes for known toxins/lysogeny-related genes, sequence similarity searches were performed against the non-redundant nr NCBI database.

Cytotoxicity towards keratinocytes

The effect of BFC-1 on the proliferation of primary neonatal human foreskin keratinocytes was evaluated in triplicate, using the trypan blue dye exclusion test. Keratinocytes (nFS02-006, passage 8) were seeded at a concentration of 2500 keratinocytes per cm^2 in 24 ml Epilife^{TM} basal growth medium (Cascade Biologics, Invitrogen, Merelbeke, Belgium) in six 75 cm^2 cell culture flasks (Falcon, Becton Dickinson). One ml of BFC-1 was added to three flasks and 1 ml of sterile physiologic water was added to the three remaining flasks as a control. Flasks were incubated at 37°C, 5% CO_2 and a relative humidity (RH) of 95% for 5 days without medium change. Three ml of 0.025% trypsin in 0.01% EDTA solution (Lonza, Verviers, Belgium) was added to the flasks. After incubation at 37°C in 5% CO_2 and 95% RH for 4 min, three millilitres of 0.025% trypsin inhibitor (Sigma-Aldrich) was added and mixed by pipetting. The keratinocyte suspension was collected in a 15 ml tube (Falcon, Becton Dickinson) and centrifuged at 170 g for 10 min. The pellet was resuspended in 5 ml Epilife^{TM} basal growth medium. Subsequently, 100 μl of cell suspension was mixed with 100 μl of a trypan blue staining solution (Biochrom AG, Berlin, Germany) and the living (colourless) and dead (blue) cells were counted using a Bürker cell counting chamber and an inverted microscope (TMS-F, Nikon, Belgium). The mean ratio of dead keratinocytes versus the total number of keratinocytes, expressed in %, compared to the control group, was taken as a measure for the cytotoxicity of BFC-1.

Stability at 4°C

The stability of a dedicated batch of BFC-1 was monitored on a monthly basis, by determining the titre of each of the three bacteriophages after storage at 4°C. Taking into account test result deviations, inherent to bacteriological methods, titres within a range of 1.10^8 to 1.10^{10} pfu/ml confer retention of activity and thus stability.

Stability after spraying

One ml of BFC-1 was aspired in a 2 ml syringe. A spraying head with pore size of 300 μm was fixed onto the syringe and the total volume of cocktail in the syringe was sprayed into a 50-ml tube. Phage titres were determined prior to and after spraying by the double agar overlay method.

Batch record file

A batch record file was compiled and checked for conformity to the product information file by an industrial pharmacist. Principal aspects contained in the batch record file include:

- the positive advice of the leading ethical committee that approved the BFC-1 clinical study;
- the BFC-1 product information file, which describes, in detail, the production process and the characteristics of BFC-1;
- the material transfer and confidentiality disclosure agreement signed by all partners;
- the filled out working instructions describing all BFC-1 production steps;
- the quality and analysis certificates of all products, materials and equipment (e.g. bench flow), used in the production of BFC-1;
- the results and analysis certificates of all final quality control tests; and
- the labels of the BFC-1 vials.

Clinical application

Prior to patient application, 3 ml of BFC-1 was aspirated from a 'single use only' vial (**Figure 2**) using a sterile 5 ml

Figure 2. The final product, a defined bacteriophage cocktail.
doi:10.1371/journal.pone.0004944.g002

syringe (B. Braun) with a sterile 20 G×2″ needle (Terumo Europe, Leuven, Belgium). The needle was replaced by a sterile spray nozzle (actuator V04.1313 BC/NR with micromist insert V06.203, Robertpack Engineering B.V., Zwolle, The Netherlands). BFC-1 was sprayed on the infected burn wound (**Figure 3**).

Results

Selection and host range activity of therapeutic bacteriophages

The activity of 82 *P. aeruginosa* and 8 *S. aureus* bacteriophages from the collections of the Eliava Institute for Bacteriophage,

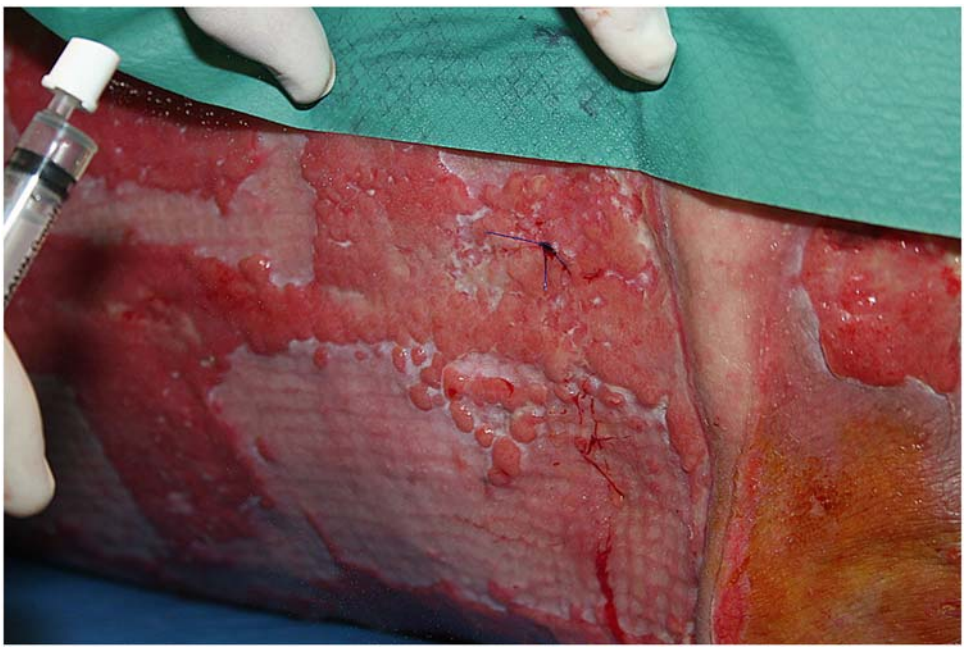


Figure 3. Application of BFC-1 on an infected burn wound using a syringe spray.
doi:10.1371/journal.pone.0004944.g003

Table 1. Characteristics of the three lytic bacteriophages present in BFC-1.

Phage	14/1	PNM	ISP
Host species	*P. aeruginosa*	*P. aeruginosa*	*S. aureus*
Initial source	Sewage water	Mtkvari River	Unknown
Initial place of isolation	Regensburg, Germany	Tbilisi, Georgia	Tbilisi, Georgia
Initial date of isolation	2000	1999	1920–1930
Isolated by	V. Krylov (SIGSIM)	N. Lashki & M. Tediashvili (EIBMV)	from ISP[a]
Serogroup	E	PT5, PNC101	ISP
Genome size (kb)	66.1	42.4	120
Family of *Caudovirales*	*Myoviridae* A1	*Podoviridae* C1	*Myoviridae* A1
Host range activity (%)			
All strains	37	44	91
BWC strains	83	96	100

[a]ISP: Intravenous Staphylococcal Phage (ISP) preparation produced by Eliava IBMV. Phage ISP was isolated from this preparation in the 1970s.
doi:10.1371/journal.pone.0004944.t001

Microbiology and Virology (EIBMV), Tbilisi, Georgia and the State Institute of Genetics and Selection of Industrial Microorganisms (SIGSIM), Moscow, Russia was determined against a total of 113 *P. aeruginosa* and 99 *S. aureus* strains, isolated from different clinical and environmental habitats across the world. The complete data on used strains and phages is shown in **Table S1**.

Eight bacteriophages (PT6, PT8, PNM, 14/1, 9/3, F77, PL and ISP) were selected for further matching against 23 *P. aeruginosa* and 17 *S. aureus* strains, recently isolated from patients at the Burn Centre of the Queen Astrid Military Hospital in Brussels. The data on strains and phages tested are shown in **Table S2**. This resulted in the selection of three bacteriophages that exhibited a large host range activity, specific for the burn wound isolates: PNM, 14/1 and ISP (**Table 1**).

P. aeruginosa bacteriophage PNM, a member of the *Podoviridae* family, was isolated in 1999 from the Mtkvari river in Tbilisi, *P. aeruginosa* bacteriophage 14/1, a member of the *Myoviridae* family, was isolated by Victor Krylov in 2000 from sewage water in Regensburg, Germany and *S. aureus* bacteriophage ISP, also a member of the *Myoviridae*, was isolated from the Intravenous Staphylococcal Phage (ISP) preparation produced by the EIBMV in the 1970s. ISP was initially isolated in the 1920s from an unknown source in Tbilisi, Georgia.

Quality control parameters verified for this cocktail included sterility, pyrogenicity and pH stability, evaluated as described in the materials and methods section and further specified in **Table 2**.

Transmission electron microscopy

Microscopy confirmed that BFC-1 only contained bacteriophage particles with the expected morphology (**Figure 4**) and the expected target strain activity.

Different types of particles were observed consisting of a non-enveloped head with icosahedral symmetry and a tail with helical

Table 2. Summary of the quality control test results.

Test	Specifications	Method	BFC-1 test result
pH	6.0–8.0	pH test (EP6)	7.0 **(conform)**
Pyrogenicity	≤1.15°C temperature rise	Intravenous injection in 3 rabbits (EP6)	0.5°C **(conform)**
Sterility	Sterile	Membrane filtration method (EP6)	Sterile **(conform)**
Cytotoxicity	No cytotoxicity, no growth inhibition, no morphology changes	Co-culture with human keratinocytes	No cytotoxicity **(conform)**, **n**o growth inhibition **(conform)**, no morphology changes **(conform)**
Activity (titre)	log(8)–log(10) pfu/ml	Bacteriophage titration	8 log(8)–log(9) pfu/ml **(conform)**
Morphology of the bacteriophages	2 *Myoviridae* and 1 *Podoviridae*	Transmission electron microscopy	2 *Myoviridae* and 1 *Podoviridae* **(conform)**
Recognition of targeted bacteria	Specific recognition	Transmission electron microscopy	One *Myo-* and one *Podoviridae* member recognize and kill *P. aeruginosa*. The other *Myoviridae* member recognizes and kills *S. aureus* **(conform)**
Phage intactness Absence of cellular debris	Intact, pure	Transmission electron microscopy	Pure **(conform)**
Temperate bacteriophages in host strains	Total absence	Mitomycin C induction of temperate bacteriophages	Total absence **(conform)**
Lytic nature	Lytic	DNA sequence and proteome analysis	Lytic **(conform)**
Presence of toxic proteins	No toxic proteins	DNA sequence and proteome analysis	No predicted toxic proteins **(conform)**

doi:10.1371/journal.pone.0004944.t002

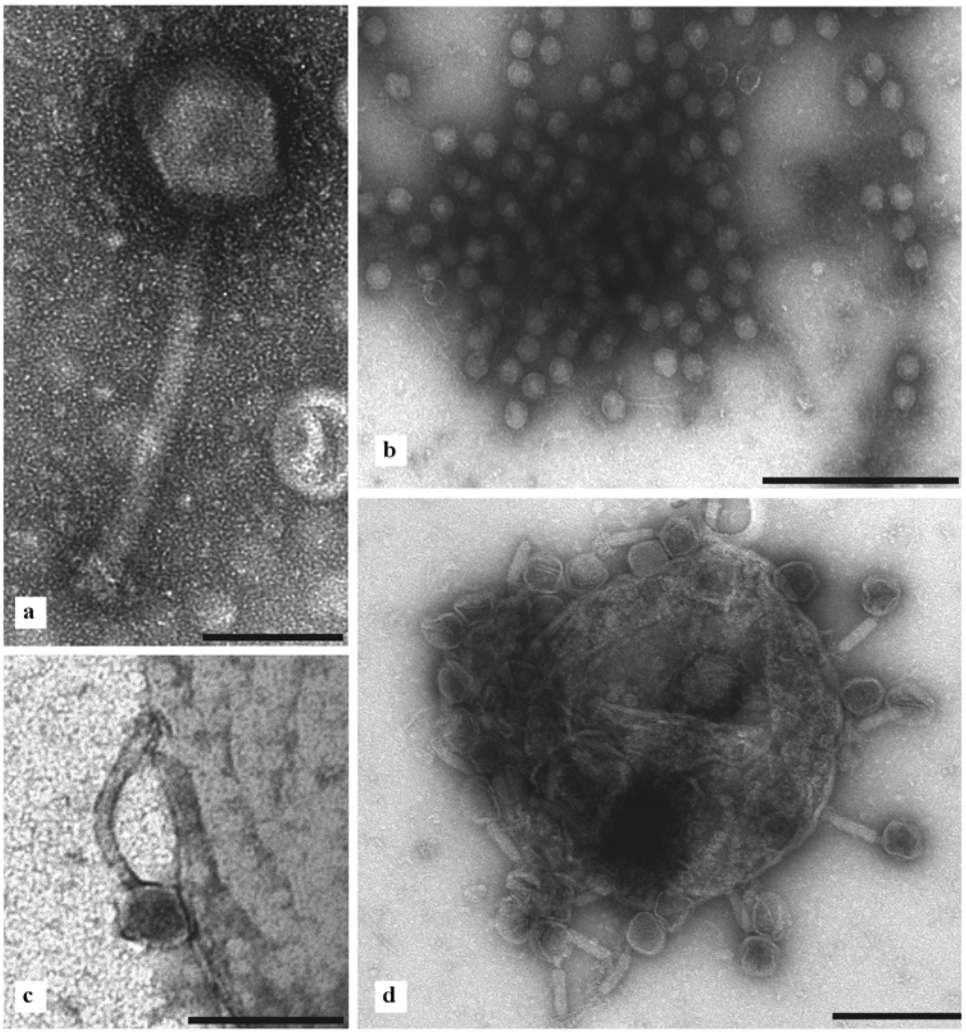


Figure 4. BFC-1 transmission electron micrographs. a) *S. aureus* bacteriophage ISP, a member of the *Myoviridae* family. Bar: 100 nm. b) PNM bacteriophages (*Podoviridae*) freed from a burst *P. aeruginosa* bacterium. Bar: 500 nm. c) Bacteriophage 14/1 attaching to the *P. aeruginosa* cell wall. Bar: 200 nm. d) ISP bacteriophages (*Myoviridae*) attached to *S. aureus*. Bar: 500 nm.
doi:10.1371/journal.pone.0004944.g004

symmetry. These characteristics are consistent with the attributes of the bacteriophage order *Caudovirales*.

One type of particles had an isometric hexagonal head with a diameter of 60 nm and a straight, short, thick, non-contractile tail built of stacked non-banded rings. The tail had a length of 10 nm and a width of approximately 8 nm. Subterminal fibers were short and difficult to visualize. These characteristics attribute this type of bacteriophages to the *Podoviridae* family.

Two morphotypes of *Myoviridae* could be distinguished, based on the respective sizes of the head and the length of the (non-contracted) tail. One population had a head diameter of approximately 78 nm and a tail length of 100 nm, whereas a second morphotype had a head diameter of approximately 90 nm and a tail length of approximately 175 nm.

When BFC-1 was combined with cultured *S. aureus* and *P. aeruginosa* bacteria, bacteriophages from the *Podoviridae* and the *Myoviridae* adsorbed to (and killed) their respective host.

The material observed in BFC-1 was pure, consisting almost entirely of (exculsively lytic) bacteriophages, or bacteriophage-derived material (isolated tails, heads, etc). No other etiological agents or residual bacteria were observed.

DNA sequencing and *in silico* proteomic analysis

The complete genome sequences of the two *P. Aeruginosa* infecting phages 14/1 and PNM were determined by a combination of shotgun sequencing and primer walking as described in Experimental Procedures. The genome of 14/1 comprises 66.2 kb and has a G+C content of 55.6%, which is significantly lower than that of its host (66.6%).

Sequence analysis showed that bacteriophage 14/1 is a close relative of bacteriophage F8 (66 kb) [31], having an overall DNA identity of 87% (total average), spread throughout the genome. Bacteriophage F8 is one of the original Lindberg *Pseudomonas* typing phages. Among the 90 predicted gene products of 14/1 (or corresponding homologsin F8 or F8-like bacteriophages like BcepF1 or BcepB1A) no toxic proteins are present and the genome does not contain a recognizable integrase gene, corroborating the lytic nature of these bacteriophages. Indeed, an initial screen revealed a single, potentially toxic gene (VirE) within the genome of phage 14/1 (NC_011703). However, the BCep1 VirE homologue was recently connected to the prim-pol primase superfamily of DNA polymerases, implying a role in phage DNA replication for VirE, rather than promoting host pathogenicity.

The genome of PNM is 42.7 kb and with its 62.3% it approximates the host G+C content.

The bacteriophage PNM genome shows close homology to ϕKMV, with only minor (single nucleotide) differences between PNM and ϕKMV, located in the DNA replication and structural region (average homology >95%). Differences are most obvious in the early region (80–90% DNA homology), which is consistent with the known genomic variations between the members of the ϕKMV-like bacteriophages [32]. To date, proteomic characterization of ϕKMV-like viruses has not revealed any toxic gene products present in these bacteriophages [32,33], indicating their absence in PNM.

For bacteriophage ISP, the 816 sequencing runs on shotgun clones yielded a total of 120 kb of unique sequences, 100 kb of which is contained within contigs. This represents about 86% of the predicted genome length of about 140 kb. Preliminary analysis of the ISP genome data suggests that this bacteriophage is almost identical to *S. aureus* bacteriophage G1 (over 99% DNA homology). The homology of bacteriophage ISP to bacteriophages G1 and K is interesting from a therapeutic perspective, since these bacteriophages have been used in several clinical settings and animal studies [34,35].

Physicochemical properties of BFC-1

The pH of BFC-1 was 7.0, which is within specifications (6.0–8.0 pH).

Since ten percent of the BFC-1 production was found to be sterile as assessed by the membrane filtration method, the product was considered to conform to the European Pharmacopoeia standards (EP6).

The monthly titration of the separate bacteriophages in BFC-1 showed a conservation of 100% of the initial activity (1.10^9 pfu/ml) for at least 12 months and further testing is ongoing.

Toxicity and pyrogenicity of BFC-1

No cytotoxicity towards human neonatal foreskin keratinocytes was observed. The addition of BFC-1 to the culture medium had no impact on the viability of the keratinocytes (**Table 3**). The proliferation rate of the keratinocytes, represented by the mean cell number after 5 days of culture (**Table 3**), was not inhibited, and the morphology of the keratinocytes was not altered by the bacteriophages. Keratinocyte cultures, with and without BFC-1, reached 80–90% confluence after 5 days of culture whilst maintaining a normal morphology.

Since the sum of the increase of body temperature after injection of 1.2 ml of BFC-1 in the three rabbits was 0.5°C, which is much lower than the allowed increase of 1.15°C, the product was found to conform to the European Pharmacopoeia standards (EP6).

Table 2 summarizes the tests, specifications, methods and results of the final quality control.

Table 3. Results of cytotoxicity testing.

Without BFC-1		
Culture flask	**Cell number (log6)**	**Viability (%)**
1	2.00	90.9
2	2.23	89.3
3	2.10	85.1
Mean:	2.11	**88.5**
Standard deviation:	1.17	3.0
With BFC-1		
4	1.90	93.4
5	2.30	90.8
6	2.33	89.7
Mean:	2.18	**91.3**
Standard deviation:	2.41	1.9

doi:10.1371/journal.pone.0004944.t003

Discussion

Phage therapy has the potential to be one of the promising alternatives/complements to antibiotics. In the past, this approach was often not as effective as hoped. Reasons for this included the empirical use of poorly characterised crude bacteriophage preparations. In addition, the clinical application of bacteriophages for treatment of infections of humans in modern western medicine is stuck in a vicious regulatory circle [14]. Under the current regulatory framework, bacteriophages do not exist because of the lack of clinical trials - yet to perform these trials one needs a regulatory existence. As such, the development of a well-characterized bacteriophage preparation using GMP (Good Manufacturing Practices)-like procedures was warranted for scientific and medico-legal reasons.

All products used in the production of BFC-1, were certified or accompanied by an adequate certificate of analysis and were fully compatible with the topical application on burn wound patients. In addition, all equipment (e.g. pipettes, bench flow and incubators) used in the production of BFC-1 was calibrated and certified. Apart from the bacteriophages (PNM, 14/1 and ISP), BFC-1 is composed of sterile and apyrogenic water (*aqua ad injectabilia* as required by the European Pharmacopoeia) as solvent, supplemented with 0.9% w/w NaCl for adaptation to physiologic osmotic strength.

The minimal bacteriophage titres required for the intended clinical applications are unknown. Bacterial loads of 10^5 bacteria per g wound tissue were shown to confer a septicaemic risk [36]. Our choice of 10^9 pfu/ml of each bacteriophage, applied in doses of 1 ml per 50 cm^2 wound bed, should result in concentrations of at least 100 bacteriophages for each target bacterium.

Since it cannot be excluded that BFC-1 also contains traces of the initial bacterial growth medium and since traditional growth media for bacteria contain animal extracts (implying a risk of transmission of infectious agents such as BSE), it was decided to use a bacterial growth medium certified to be free of animal proteins. The most important medium remnants in BFC-1 are soy hydrolysate and yeast extract, inherent components of the Select APS LB Broth Base used for bacterial growth and bacteriophage production. The theoretical final concentrations of these remnants, before endotoxin removal, are 25 and 125 μg/ml of BFC-1 respectively.

Phage therapy faced several problems often due to an inadequate preparation methodology. Purification, removal of endotoxins and pyrogenic substances, stability and pH control of the preparation were rather problematic in the past [6]. Endotoxins possess a high degree of toxicity, and their removal is essential for safety in antibacterial bacteriophage therapy [37]. BFC-1 was easily and successfully purified from endotoxins using a commercially available, column endotoxin purification kit, which is based on the principles of affinity chromatography. The high endotoxin affinity ligand of the EndoTrap® Blue affinity matrix is proteinaceous and derived from a bacteriophage. It is

not an antibody, and is covalently immobilized on agarose beads in order to ensure negligible leakage.

Accredited laboratories, able to deliver certified results, performed the final quality control tests such as determination of pH, pyrogenicity and sterility, classically required in clinical studies. An in-house cytotoxicity test was performed. For endotoxin testing, the *Limulus* Amoebocyte Lysate (LAL) assay is the regulatory first line test (EP6, chapter 2.6.14/11.2). The reference laboratory to which the cocktail was sent for pyrogenicity testing first attempted to apply the LAL assay, but the phages appeared to interfere with this test. The rabbit pyrogenicity test can be used only when there is interference with the endotoxin test, which was the case here. In fact the pyrogenicity test encompasses all pyrogens and not only endotoxins. We applied the real volumes that were to be applied on patients and took the largest safety factor for acceptability of the phage cocktail.

In theory, there is no need to ascertain the absence of pyrogens from products, which are not intravenously/parenterally administered. However, we worked with a product that during its production process was in close contact with bacteria and that by application to a burn wound could diffuse partially into the blood stream.

The pH is a homeostasis and stability indicator and thus an important parameter of a therapeutic product. A pH between 6.0 and 8.0 guarantees the stability of infectivity of the bacteriophages [38] and is compatible with the wound bed physiology [39]. The pH 7.0 of BFC-1 (ideal for stable storage) is suitable for topical use on burn wounds.

According to guideline Q5C (Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products), published by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the manufacturer should propose a stability indicating profile to ensure that changes in the identity, purity and potency of the product will be detected. Since BFC-1 consists of three bacteriophages suspended in a physiological solution and is stored at 2–8°C in 3 ml 'single use only' vials, identity and purity are not considered likely to alter. In this particular case, the parameter that is thus proposed to profile the stability characteristics of BFC-1 is the activity or potency of the sole active components of BFC-1, the bacteriophages PNM, 14/1 and ISP. The slightest deterioration of one or all of the bacteriophages will immediately result in a decrease of the capacity to achieve its intended effect (= activity or potency) of BFC-1. This capacity was measured through titration of the bacteriophages against the host strains that were used to propagate the phages. BFC-1 was shown to maintain 100% of its initial activity after storage at 4°C for at least one year. Further follow-up of stability testing is ongoing.

Since temperate bacteriophages are known to transfer antibiotic resistance and virulence genes from one bacterium to another, a process known as lysogenic conversion, the absence of temperate bacteriophages from the host bacteria used in the production of BFC-1 had to be confirmed. The chemical induction test suggested an absence of temperate bacteriophages and the DNA sequence and proteomic analysis confirmed the lytic character of the bacteriophages and the absence of toxin genes. Transmission electron microscopy confirmed the phage particle intactness and expected morphology as well as the absence of cellular debris.

The host specificity of the phages could be established by means of electron microscopy as well.

Data documenting the BFC-1 production process and the certified quality control tests on the final product were compiled into a batch record file. An industrial pharmacist certified the conformity of this file to the product information file.

BFC-1 is currently evaluated in a clinical trial, which was approved by the Ethical Committee of the "Universitair Ziekenhuis" of the "Vrije Universiteit Brussel" and started in October 2007. To date, BFC-1 has been applied topically (**Figure 3**) on the infected burn wounds of eight patients. No adverse events were observed.

This is, to our knowledge, the first detailed description of a quality-controlled small-scale production of a bacteriophage preparation, leading to a safety trial in burn wound patients, which was approved by a leading Belgian ethical committee. The aim of this manuscript was therefore not to produce a commercial product, or to assess regulatory aspects of phage therapy, but merely implementing a small step in the further evaluation of phage therapy in Western medicine.

Together with European experts, we are currently in the process of creating a discussion platform that could act as one of the interlocutors with the regulatory authorities (e.g. EMEA) for the creation of a specific regulatory framework and appropriate production standards for phage therapy. Another, equally important objective is to help provide the necessary studies to enable a coherent use of phage therapy.

Supporting Information

Table S1 Activity of phages against 113 *P. aeruginosa* and 99 *S. aureus* strains, isolated from different clinical and environmental habitats across the world.
Found at: doi:10.1371/journal.pone.0004944.s001 (0.25 MB XLS)

Table S2 Activity of phages against 23 *P. aeruginosa* and 17 *S. aureus* strains, recently isolated from patients at the Burn Centre of the Queen Astrid Military Hospital in Brussels.
Found at: doi:10.1371/journal.pone.0004944.s002 (0.03 MB XLS)

Acknowledgments

The authors wish to thank Mr. William Anderson for reviewing this manuscript.

Author Contributions

Conceived and designed the experiments: MM JPP GV NC MT VK RL GV MZ DDV MV. Performed the experiments: MM NL TG JM WM GV PDC TR. Analyzed the data: MM JPP GV NC MT NL TG VK JM LVP RL GV WM GV PDC TR SJ MZ DDV MV. Contributed reagents/materials/analysis tools: MM JPP GV NC MT VK JM RL SJ DDV MV. Wrote the paper: MM JPP GV NC MT VK JM LVP RL MZ DDV MV.

References

1. Altoparlak U, Erol S, Ackay MN, Celebi F, Kadanali A (2004) The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. Burns 30: 660–664.
2. Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006) Burn wound infections. Clin Microbiol Rev 19: 403–434.
3. Pirnay J-P, De Vos D, Cochez C, Bilocq F, Pirson J, et al. (2003) Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J Clin Microbiol 41: 1192–1202.
4. d'Hérelle F (1917) Sur un microbe invisible antagoniste des bacilles dysentériques. Acad Sci Ser D: 165, 373.

5. Levy SB, Marschal B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10: S112–S129.
6. Brüssow H (2007) Phage therapy: the Western perspective. In: McGrath S, van Sinderen D, eds. Bacteriophage Genetics and Molecular Biology. Norfolk: Caister Academic Press. pp 159–192.
7. Chanishvili N, Chanishvili T, Tediashvili M, Barrow PA (2001) Phages and their application against drug-resistant bacteria. J Chem Technol Biotechnol 76: 689–699.
8. Górski A, Borysowski J, Miedzybrodzki R, Weber-Dabrowska B (2007) Bacteriophages in medicine. In: Mc Grath S, van Sinderen D, eds. Bacteriophage Genetics and Molecular Biology. Norfolk: Caister Academic Press. pp 126–158.
9. Krylov VN (2001) Phage therapy in terms of bacteriophage genetics: hopes, prospects, safety, limitations. Russian J Genetics 37: 869–887.
10. Merril CR, Scholl D, Adhya SL (2003) The prospect for bacteriophage therapy in Western medicine. Nat Rev Drug Discov 2: 489–497.
11. Sulakvelidze A, Kutter E (2005) Bacteriophage therapy in humans. In: Kutter E, Sulakvelidze A, eds. Bacteriophages: Biology and Applications. Boca Raton: CRC Press. pp 381–436.
12. Calendar R, Abedon S, eds (2006) The bacteriophages. Oxford: Oxford Univ Press. 746p.
13. Villareal LP (2005) Overall issues of virus and host evolution. In: Villareal LP, ed. Viruses and the Evolution of Life. Washington: ASM Press. pp 1–28.
14. Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, et al. (2007) European regulatory conundrum of phage therapy. Future Microbiol 2: 485–491.
15. Babalova EG, Katsitadze KT, Sakvarelidze LA, Imniashvili NS, Sharashidze TG, et al. (1968) Preventive value of dried dysentery bacteriophage. Zh Mikrobiol Epidemiol Immunobiol 2: 143–145.
16. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, et al. (2002) Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect Immun 70: 204–210.
17. Bruttin A, Brüssow H (2005) Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob Agents Chemother 49: 2874–2878.
18. Capparelli R, Ventimiglia I, Roperto S, Fenizia D, Iannelli D (2005) Selection of an *Escherichia coli* O157:H7 bacteriophage for persistence in the circulatory system of mice infected experimentally. Clin Microbiol Infection 12: 248–253.
19. Chibani-Chennoufi S, Sidoti J, Brüttin A, Kutter E, Sarket, et al. (2004) *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. Antimicrob Agents Chemother 48: 2558–2569.
20. McVay CS, Velasquez M, Fralick JA (2007) Phage therapy of *Pseudomonas aeruginosa* infections in a mouse burn wound model. Antimicrob Agents Chemother 51: 1934–1938.
21. Smith HW, Huggins MB (1982) Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. J Gen Microbiol 128: 307–318.
22. Smith HW, Huggins MB (1983) Effectiveness of phage treatment in experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J Gen Microbiol 129: 2659–2675.
23. Smith HW, Huggins MB, Shaw KM (1987) The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. J Gen Microbiol 133: 1111–1126.
24. Soothill JS (1992) Treatment of experimental infection of mice with bacteriophages. J Med Microbiol 37: 258–261.
25. Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, et al. (2007) Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. Antimicrob Agents Chemother 51: 446–452.
26. Wills QF, Kerrigan C, Soothill JS (2005) Experimental bacteriophage protection against *Staphylococcus aureus* abscess in a rabbit model. Antimicrob Agents Chemother 49: 1220–1221.
27. Adams MH (1959) Assay of phages by the agar layer method. In: Adams MH, ed. Bacteriophages. New York: Interscience Publishers. pp 450–451.
28. Carlson K (2005) Methodological Approaches. In: Kutter E, Sulakvelidze A, eds. Bacteriophages: Biology and Applications. Boca Raton: CRC Press. pp 437–490.
29. Imberechts H, Wild P, Charlier G, De Greve H, Lintermans P, et al. (1996) Characterization of F18 fimbrial genes *fedE* and *fedF* involved in adhesion and length of enterotoxemic *Escherichia coli* strain 107/86. Microb Pathog 21: 183–192.
30. Miller RV (1998) Methods for enumeration and characterization of bacteriophages from environmental samples. In: Burlage RS, Atlas R, Stahl D, Geesey G, Sayer GS, eds. Techniques in Microbial Ecology. New York: Oxford University Press. pp 218–235.
31. Kwan T, Liu J, DuBow M, Gros P, Pelletier J (2006) Comparative genomic analysis of 18 *Pseudomonas aeruginosa* bacteriophages. J Bacteriol 188: 1184–1187.
32. Ceyssens PJ, Lavigne R, Mattheus W, Chibeu A, Hertveldt K, et al. (2006) Genomic analysis of *Pseudomonas aeruginosa* phages LKD16 and LKA1: establishment of the phiKMV subgroup within the T7 supergroup. J Bacteriol 188: 6924–6931.
33. Lavigne R, Burkal'tseva MV, Robben J, Sykilinda NN, Kurochkina LP, et al. (2003) The genome of bacteriophage phiKMV, a T7-like virus infecting *Pseudomonas aeruginosa*. Virology 312: 49–59.
34. O'Flaherty S, Coffey A, Edwards R, Meaney W, Fitzgerald GF, et al. (2004) Genome of staphylococcal phage K: a new lineage of *Myoviridae* infecting gram-positive bacteria with a low G+C content. J Bacteriol 186: 2862–2871.
35. Gill JJ, Pacour JC, Carson ME, Leslie KE, Griffiths MW, et al. (2006) Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. Antimicrob Agents Chemother 50: 2912–2918.
36. Perez-Cappelano R, Manelli JC, Palayret D, Carlin G, Echinard C, et al. (1976) Evaluation of the septicaemic risk by a quantitative study of the cutaneous flora in patients with burns. Burns 3: 42–45.
37. Boratynski JD, Syper D, Weber-Dabrowska B, Kusiak-Szelachowska M, Pozniak G, et al. (2004) Preparation of endotoxin-free bacteriophages. Cell Mol Biol Lett 9: 253–259.
38. Kerby GP, Gowdy RA, Dillon ES, Dillon ML, Csâky TZ, et al. (1949) Purification, pH stability and sedimentation properties of the T7 bacteriophage of *Escherichia coli*. J Immunol 63: 93–107.
39. Schneider LA, Korber A, Grabbe S, Dissemond J (2007) Influence of pH on wound-healing: a new perspective for wound-therapy. Arch Dermatol Res 298: 413–420.
40. Merabishvili M, Verhelst R, Glonti T, Chanishvili N, Krylov V, et al. (2007) Digitized fluorescent RFLP analysis (fRFLP) as a universal method for comparing genomes of culturable dsDNA viruses: application to bacteriophages. Res Microbiol 158: 572–581.

2.3 The launch of a bacteriophage therapy safety trial (Study 3)

2.3.1 Experimental bacteriophage therapy of burn wound infection: difficult first steps

T. Rose, G. verbeken, D. De Vos, M. Merabishvili, M. Vaneechoutte, R. Lavigne, S. Jennes, M. Zizi, J.P. Pirnay

Int J Burn Trauma. 2014; 4(2):66-73

International scientific journal, peer-reviewed

2.3.2 Bacteriophages for the treatment of severe infections: a ‘new’ option for the future?

D. De Vos, G. Verbeken, T. Rose, S. Jennes, J.P. Pirnay

EWMA Journal. 2012; 12(2):23-28

International scientific journal

2.3.1 Experimental bacteriophage therapy of burn wound infection: difficult first steps

T. Rose, G. verbeken, D. De Vos, M. Merabishvili, M. Vaneechoutte, R. Lavigne, S. Jennes, M. Zizi, J.P. Pirnay

Int J Burn Trauma. 2014; 4(2):66-73

International scientific journal, peer-reviewed

Int J Burn Trauma 2014;4(2):66-73
www.IJBT.org /ISSN:2160-2026/IJBT0001948

Original Article

Experimental phage therapy of burn wound infection: difficult first steps

Thomas Rose[1], Gilbert Verbeken[2], Daniel De Vos[2], Maya Merabishvili[2,3,4], Mario Vaneechoutte[4], Rob Lavigne[5], Serge Jennes[1], Martin Zizi[6], Jean-Paul Pirnay[1]

[1]Burn Wound Center, Queen Astrid Military Hospital, Bruynstraat 1, B-1120 Brussel, Belgium; [2]Laboratory for Molecular and Cellular Technology, Burn Wound Center, Queen Astrid Military Hospital, Bruynstraat 1, B-1120 Brussel, Belgium; [3]Eliava Institute of Bacteriophage, Microbiology and Virology, 3, Gotua street, Tbilisi 0160, Georgia; [4]Laboratory of Bacteriology Research, University of Ghent, De Pintelaan 185, B-9000 Gent, Belgium; [5]Laboratory of Gene Technology, Katholieke Universiteit Leuven, Kasteelpark Arenberg 21, B-3001 Leuven, Belgium; [6]Department of Physiology, Vrije Universiteit Brussel, Laerbeeklaan 101, B-1090 Brussel, Belgium

Received August 18, 2014; Accepted September 8, 2014; Epub October 26, 2014; Published October 30, 2014

Abstract: Antibiotic resistance has become a major public health problem and the antibiotics pipeline is running dry. Bacteriophages (phages) may offer an 'innovative' means of infection treatment, which can be combined or alternated with antibiotic therapy and may enhance our abilities to treat bacterial infections successfully. Today, in the Queen Astrid Military Hospital, phage therapy is increasingly considered as part of a salvage therapy for patients in therapeutic dead end, particularly those with multidrug resistant infections. We describe the application of a well-defined and quality controlled phage cocktail, active against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, on colonized burn wounds within a modest clinical trial (nine patients, 10 applications), which was approved by a leading Belgian Medical Ethical Committee. No adverse events, clinical abnormalities or changes in laboratory test results that could be related to the application of phages were observed. Unfortunately, this very prudent 'clinical trial' did not allow for an adequate evaluation of the efficacy of the phage cocktail. Nevertheless, this first 'baby step' revealed several pitfalls and lessons for future experimental phage therapy and helped overcome the psychological hurdles that existed to the use of viruses in the treatment of patients in our burn unit.

Keywords: Phage therapy, burn wound, infection, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, antibiotic resistance

Introduction

Multidrug resistance, first reported in the 1970s, has become a major threat to the progress made in infection control worldwide. Each year in the EU, an estimated 25,000 patients die from infections with multidrug-resistant (MDR) bacteria [1]. Also in burn units, a large number of infections are virtually untreatable. Whereas *Staphylococcus aureus* remains a common early colonizer of burn wounds, *Pseudomonas aeruginosa* is known as the most common cause of life-threatening infection in burn patients [2, 3]. Both bacteria, but especially *P. aeruginosa,* are known for their intrinsic and acquired resistance to many antibiotics. Persistent multidrug-resistant *P. aeruginosa* strains have frequently been reported to cause nosocomial outbreaks of infection in burn units [4, 5].

Bacteriophages (phages) are (among) the most abundant and ubiquitous organisms on Earth and are the natural controllers of bacteria. They are the 'viruses' of the bacteria and are able to lyse, among others, strains of *S. aureus* or *P. aeruginosa*, irrespective of the antibiotic susceptibility of these strains. As such, they may offer an independent means of infection treatment, which can be combined or alternated with antibiotic therapy and may enhance our abilities to treat bacterial infections successfully [6]. Since the 1920s, phages have been used to treat all sorts of bacterial infections in Eastern Europe and the former USSR States, with the Eliava Institute in Tbilisi (Georgia) as

one of the key centers [7]. The advent of antibiotics, which exhibited a broader spectrum of activity and which could be produced easier in large quantities (i.e. in a commercially more profitable manner), forced phage therapy to the margins of Western medicine. With the worldwide spreading of MDR bacteria, however, the therapeutic use of phages is going through a renaissance in the Western world [8].

The few burn wound related phage therapy papers in the scientific literature [9-17] suggest that phages could have the potential to control bacterial burn wound infection.

Phages were shown to be able to rescue burned mice from infection caused by *P. aeruginosa* and *Klebsiella pneumonia* [9, 10]. In 1990, in Egypt, 30 patients with burn wounds were treated during 5-17 days with between 15 and 45 phage-saturated dressings [11]. The clinical success ratio was difficult to assess because of the lack of validated controls, but the mere fact that not-endotoxin-purified phages had been applied massively to burn wounds, without reporting adverse effects, could be indicative for their intrinsic harmlessness. Soothill and colleagues showed that in a test population of 14 guinea pigs with excised burn wounds to which 6 x 10^5 cfu/ml of *P. aeruginosa* and 1.2 x 10^7 *P. aeruginosa* BS24 phages were applied simultaneously and upon which the excised tissue was replaced, 6 out of 7 phage treated grafts were not rejected, whereas all 7 of non phage treated grafts failed [12]. Weber-Dabrowska *et al*. reported the treatment of 49 burn wounds in human patients, infected with *P. aeruginosa*, *S. aureus*, *Escherichia coli*, *Klebsiella* and/or *Proteus*. Forty-two patients fully recovered and the condition of the remaining 7 patients improved markedly [13]. A 2005 publication addressed the treatment of local radiation injuries in two individuals, using a novel biodegradable preparation capable of sustained release of phages and ciprofloxacin [14]. The same product was applied in Georgia on 22 patients with infected venous static ulcers and other poorly healing wounds, after standard therapy had failed [15]. Seventy percent of the patients showed full recovery after a period ranging from 6 days to 15 months. In the UK, the group of Soothill reported the case of a 27-year-old male with 50% TBSA burned and excised burn wounds covered with skin grafts, which became infected with *P. aeruginosa* after several months [16]. Grafted areas broke down rapidly despite appropriate antibiotic treatment. Therefore, treatment with 'purified' phages was started. Phages multiplied in the wound and a 43 to 1200-fold increase of phages was observed. Three days after phage application, *P. aeruginosa* could not be isolated from swabs and subsequent extensive grafting was successful.

There are, however, some major obstacles hampering the clinical application of phages in Western medicine [18-22]. In the EU, discussions between small and medium-sized enterprises (SMEs) and competent authorities led to the classification of bacteriophages as human medicinal products (biologicals) regulated under the European Human Code for Medicines (Directive 2001/83/EC). A handful of companies are now struggling to take large-scale and uniform phage cocktails through the elaborate and expensive medicinal product licensing pathway. Funding for the development of phages as medicinal products is difficult to obtain, since intellectual property (IP) protection for phages (products of nature) is very fragile. In addition, we feel that in hospital settings phage therapy would better be served by small-scale productions and distributions of tailor made phage preparations [21, 22]. Finally, but not less important, the reluctance to embrace phage therapy is also linked to the false perception of viruses, with which phages are identified – often without nuance –, as 'enemies of life' [23]. As a result, in the Western world, phages for controlling microbial contamination in food and the food-processing environment are readily used, while no phage medicinal products are currently authorized for human use [24].

In 2007, we developed a well-defined phage cocktail, BFC-1, which was active against the *P. aeruginosa* and *S. aureus* strains that populated the burn wound center of the Queen Astrid Military Hospital. The quality controlled production process of BFC-1 was mainly based on our experience in producing cell and tissue autografts and allografts for human transplantation (regulated under Directive 2004/23/EC of the European Parliament and of the Council) and was published in 2009 [25].

This paper gives an account of the first application, in 2007, of BFC-1 on colonized burn wounds in the burn wound center of the Queen

Figure 1. A vial of bacteriophage cocktail BFC-1.

Astrid Military Hospital. Since in 2007 phages were not yet classified as medicinal products, these phage applications were performed within a small (9 patients, 10 single dose applications) investigator driven clinical trial (no sponsor) under the responsibility and supervision of a leading Medical Ethical Committee. The study was not designed according to one of the common phases (I, II or III) of the classical medicinal product approval process and we did not solicit for approval by any regulatory authority for future use in the general population (marketing authorization). The study was notified (by the ethical committee) to the national competent authorities and informed consent was obtained from the patients. The parties involved in this study had no commercial interests.

Materials and methods

Phage cocktail

The phage cocktail BFC-1, which was evaluated in this study, consisted of three exclusively lytic phages, designed for the treatment of *P. aeruginosa* and *S. aureus* infections in burn wound patients [25]. Based on successive selection rounds three phages were retained from an initial pool of 82 *P. aeruginosa* and 8 *S. aureus* phages, specific for the *P. aeruginosa* and *S. aureus* strains that were the most prevalent in the burn wound center of the Queen Astrid Military Hospital. This cocktail, consisting of *P. aeruginosa* phages 14/1 (*Myoviridae*) and PNM (*Podoviridae*) and *S. aureus* phage ISP (*Myoviridae*), at a concentration of 10^9 plaque forming units (pfu)/ml of each phage, was produced and purified of endotoxin according to Merabishvili *et al*. [25]. Quality controls included stability (shelf life), determination of pyrogenicity, sterility and cytotoxicity, confirmation of the absence of temperate phages and transmission electron microscopy-based confirmation of the presence of the expected virion morphologic particles as well as of their specific interaction with the target bacteria. Phage genome and proteome analysis confirmed the lytic nature of the phages and the absence of toxin-coding genes.

Patients' inclusion criteria

Nine acute burn wound patients with MDR *P. aeruginosa* and/or *S. aureus* burn wound colonization, as determined by classical bacterial culture and species identification and antibiotic susceptibility testing using the VITEK 2 system (bioMérieux) of routine burn wound swabs, were included in this study. Pregnant women and patients in critical condition (APACHE II score > 20) were not included. Only patients with burn wounds that allowed for punch biopsy sampling were included. Patients or their legal representatives were provided with relevant and understandable information regarding the study and the need to give informed consent

before they participated in the study. A no-fault (regardless of liability) compensation insurance was provided to the patients.

General trial set up

We compared the standard treatment for *P. aeruginosa* and *S. aureus* burn wound colonization with a phage treatment. Since an objective evaluation and classification of burn wounds is impossible and colonization and infection levels can vary significantly, we compared both treatments on the same colonized burn wound.

Just before the application of BFC-1, the colonized burn wound was divided into two halves. One half received the standard treatment, the other half the phage treatment with BFC-1. Two biopsies were taken by the MD in charge of the patient using a 4 mm punch biopsy needle (Labo Stiefel); one in the centre of the zone where BFC-1 was to be applied, the other in the centre of the zone where the standard treatment was to be applied. Tissue biopsies were preceded by local anesthesia (xylocaine 2%, Asta Zeneca). It was shown that infiltration with additive-free lidocaine 1% into a ring block shortly before the biopsy procedure did not affect the result of bacterial culture provided that culture was started within 2 hours [26]. Biopsy sites were sutured with green ethilon 4/0 (standard treatment site) or with blue prolene 4/0 (BFC-1 site). The MD in charge of the patient applied a single-dose of approximately 1 ml of sterile and endotoxin-purified BFC-1 per 50 cm^2 on one half of the burn wound, using a 5 ml syringe with a spray adapter (Coster®) (**Figure 1**). The other half of the burn wound was treated with antimicrobial substances according to the standard treatment protocols. Patients with suspected *P. aeruginosa* burn wound infection were administered amikacin (single initial dose of 25 mg/kg body weight) in combination with ceftazidime (single initial dose of 1 g) or meropenem (2 g/8 h) systemically. Patients with suspected *S. aureus* burn wound infection were treated with systemic vancomycin (single initial dose of 1 g) or linezolid (2 x 600 mg/d).

A digital photograph was taken of the burn wound. The entire burn wound was then covered with dressings, gauze and bandages according to the standard treatment protocols. Two to five hours later, the burn wound was uncovered. Immediately, two biopsies were taken next to (within 2 cm) the previous ones. A digital photograph was taken of the burn wound. The entire burn wound was further treated according to standard protocols. The wound biopsies were immediately weighed and collected into separate, sterile and adequately labeled microtubes (Eppendorf AG) containing 0.5 ml of sterile phosphate buffered saline (PBS) and transported to the laboratory for bacterial load determination.

The main objective of this study was to explore the hurdles that early Western phage therapy clinical trials would inevitably face. The secondary objective was to document eventual adverse events and therapeutic effects.

Determination of bacterial load

The biopsies were immediately homogenized, on ice, for 1 min at 30000 rpm, using a tissue tearer (Biospec Products, Inc.). Serial tenfold dilutions of the homogenized wound biopsy samples were spread, in triplicate, on blood agar, Manitol Salt Agar (MSA) and cetrimide agar plates (media were purchased from Becton Dickinson). Colony counts were performed after overnight incubation at 37°C. The bacterial load, expressed as colony forming units (cfu) per g tissue, was calculated for each biopsy.

Monitoring of eventual adverse events

Patient medical files were screened for adverse events, clinical abnormalities and changes in laboratory test results that could be related to the application of phages. Clinical abnormalities that were screened for included cardiovascular, renal, and respiratory complications and pain. Clinical laboratory tests included the blood formula and standard haemostasis, biochemical, pharmacological and toxicological parameters.

Informed consent and approval of a leading Medical Ethical Committee

This clinical trial was conducted with the understanding and the consent of the human subjects. The study protocol was cleared by the Medical Ethical Committee of the Vrije Universiteit Brussel (VUB), which also notified the competent authorities.

Results

Ten BFC-1 applications were performed on 9 patients (4 males, 5 females; mean age 61 years; age range, 27 to 88 years; mean TBSA burned, 30%; TBSA burned range, 6-45%). The surface of the burn wounds to which BFC-1 was administered averaged 95 cm^2 (range 25-150 cm^2) and on average 0.03 ml of BFC-1 (equating 10^7 phages) per cm^2 were applied in a single dose. The second biopsies were obtained 181 min (range 120-240 min) after BFC-1 application. Standard treatment consisted of Isobetadine® gel (Meda Pharma) (n = 4) and Mepilex® Ag (Mölnlycke) (n = 1). Five applications occurred within the time frame of a surgical procedure, prior to which the burn wounds had been washed with Hibitane (5%) and filtered tab water.

The 10 burn wounds to which BFC-1 was administered were colonized or infected with MDR (resistance to a representative of at least 3 classes of antibiotics) strains of *P. aeruginosa* (n = 7), *S. aureus* (n = 1) or both *P. aeruginosa* and *S. aureus* (n = 2). This distinction was based on the results of the most recent routine bacteriological screening of the burn wounds, associated to relevant clinical signs and biological markers. Despite the initial indications of colonization or infection, bacterial cultures of the homogenised biopsies taken before and after BFC-1 application showed only a very small bacterial load (a few colonies) in 8 of the 10 applications. In the two remaining applications bacterial loads before BFC-1 application were 10^3 and 10^8 cfu per g tissue. In all cases, the bacterial load remained unchanged, after BFC-1 application as well as after standard treatment.

No adverse events were reported and no clinical or laboratory test abnormalities related to the application of phages were observed.

Discussion

Hurdles

It was far from easy to get this small pilot study on the rail. We had to disarm a lot of resistance. This reluctance towards phage therapy was expected and was largely due to pre- and misconceptions about phage therapy. For example, we were asked to submit our phage cocktail to the National approval system for Genetically Modified Organisms (GMO), in which the safety for humans, animals and the environment is thoroughly assessed. Then, the experts of the insurance company that was asked to provide the no-fault compensation insurance assimilated phages with viruses and consequently assigned our modest experiment to risk class 5 (on a scale from 1 to 7), which resulted in a relatively high premium. Some editors and reviewers, who evaluated a former paper describing the quality controlled production of our phage cocktail, asked for conventional pharmaceutical tests and clinical trials, which take many years and cost millions of euros. They reckoned phages should be considered as classical drugs. Although phages are therapeutic agents, we disagree on the fact they have to be considered as classical drugs. Phages are evolving natural controllers of bacteria. If one were to consider them as a stable 'drug', and apply the whole regulatory framework thereof, their composition and characteristics are not meant to vary. Unfortunately, bacteria are expected to escape such 'stable' phage preparations and the real power of the use of phages would be lost. The real added value of phages as antimicrobials relies on the possibility to generate certified phage preparations on faster time scales than those common for classical medicinal products. Then and only then will we have a 'new' powerful and sustainable tool in the fight against bacterial diseases. Hence, if they are to be successful, phages cannot be considered as classical molecules and will thus need a dedicated regulatory framework with adequate and realistic production and quality control requirements [20].

Pitfalls

During this study, we were confronted with some significant technical and logistic problems. We opted for biopsy samples to monitor the bacterial load of the burn wounds because they are still considered to be the gold standard by the majority of researchers [2, 27, 28]. On reflection, we found this technique to be very elaborate (e.g. necessitated local anesthesia and complex sample processing) and at some occasions we were confronted with patient and/or nursing aversion to biopsies. All in all, biopsy sampling turned out to impede the clinical trial in our burn wound center. In the future, we will likely opt for semi-quantitative swab cultures instead of quantitative biopsy cultures for

the monitoring of burn wound colonization, even if this is bound to result in less accurate quantification of burn wound colonization or infection.

The disappointing bacterial load of wound tissue at the moment of BFC-1 application was probably due to the long period (up to 7 days) between initial detection of a potential candidate with MDR *P. aeruginosa* and/or *S. aureus* burn wound colonization and the actual enrollment of this patient in the study. Major reasons for this were the delays in receiving antibiograms and obtaining informed consent. Meanwhile patients were treated with potent topical antimicrobials, dressings and systemic antibiotics. Some treatments were even applied minutes before the start of the trial. In the future, we will probably use clinical signs and biological markers of burn wound colonization or infection, instead of deferred bacteriological results, as inclusion criterion for this type of clinical trial. This would of course imply the inclusion of all *P. aeruginosa* and *S. aureus* burn wound infections, not only those with MDR strains. Finally, the sprayed BFC-1 cocktail had the tendency to run off the burn wound. The use of a suitable carrier, such as a gel or a dressing that is compatible with phage activity, seems more appropriate.

Notwithstanding these pitfalls, we were not expecting that a one-off application of 3 ml of BFC-1 on a small wound surface would generate conclusive proof of the efficacy of BFC-1.

Why publish (now)?

This study ran from the end of 2007 until 2008 and we planned to publish a report in a peer-reviewed scientific journal in the course of 2008. However, we decided to abandon the idea of a widespread scientific report of this study because it did not go as expected. We would use the experience gained during this small pilot study to set up a larger double blind study. Unfortunately, we had to put our plans on hold because meanwhile phages were classified as medicinal products and the subsequent and unattainable obligation to comply with the classical pharmaco-economical framework. We had waived publication, but colleagues from like-minded research groups were interested in the fate of our study and encouraged us to publish our experiences, as they might be helpful in convincing their competent authorities and ethical committees in approving experimental phage applications and in designing future studies. In addition, the study is increasingly mentioned (obviously without citing a peer-reviewed publication) in other scientific papers and this often without including relevant facts and details. Finally, we realized that this study had been an essential and necessary step towards the acceptance of phage therapy in our burn wound center. Since then the medical and nursing staff of our hospital has grown familiar with phages and deemed them safe for topical use on burn wounds. Today, phage therapy is increasingly part of the successful treatment of a handful of 'abandoned' patients with MDR infections, outside of a clinical trial and conform to the requirements of article 37 the Declaration of Helsinki (Unproven Interventions in Clinical Practice). Recently, the Belgian Ministry of Defense approved a feasibility study for the establishment of a dedicated phage therapy center in the Queen Astrid Military Hospital. On the first of June 2013, Phagoburn (www.phagoburn.eu), a project funded by the European Commission under the 7th Framework Programme for Research and Development was launched. It aims at evaluating phage therapy for the treatment of burn wounds infected with *Escherichia coli* and *P. aeruginosa*.

Conclusions

This small pilot study did identify some significant pitfalls and hurdles associated with phage therapy related clinical trials and broke down the psychological barriers with the healthcare team. The local topical application of bacteriophage cocktail BFC-1 on 10 burn wounds in 9 patients did not elicit any adverse events whatsoever.

Acknowledgements

We gratefully acknowledge Gunther Verween for his technical assistance. The authors would like to acknowledge the research community "Phagebiotics" (W0.022.09) grant from the FWO Vlaanderen.

Disclosure of conflict of interest

None to declare.

Address correspondence to: Jean-Paul Pirnay, Laboratory for Molecular and Cellular Technology, Burn Wound Center, Queen Astrid Military Hospital, Bruynstraat 1, B-1120 Brussel, Belgium. Tel: +32 2 264 4844; Fax: +32 2 264 4833; E-mail: jean-paul.pirnay@mil.be

References

[1] ECDC/EMEA Joint Working Group. The bacterial challenge: time to react. EMEA doc. ref. EMEA/576176/2009. Stockholm, September 2009. doi 10.2900/2518. Available from: http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf

[2] Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006; 19: 403-34.

[3] Altoparlak U, Erol S, Akcay MN, Celebi F, Kadanali A. The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. Burns 2004; 30: 660-4.

[4] Hsueh PR, Teng LJ, Yang PC, Chen YC, Ho SW, Luh KT. Persistence of a multidrug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. J Clin Microbiol 1998; 36: 1347-5.

[5] Pirnay JP, De Vos D, Cochez C, Bilocq F, Pirson J, Struelens M, Duinslaeger L, Cornelis P, Zizi M, Vanderkelen A. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J Clin Microbiol 2003; 41: 1192-202.

[6] Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 2010; 11: 69-86.

[7] Sulakvelidze A, Alavidze Z, Morris JG Jr. Bacteriophage therapy. Antimicrob Agents Chemother 2001; 45: 649-59.

[8] Thiel K. Old dogma, new tricks – 21st Century phage therapy. Nat Biotechnol 2004; 22: 31-6.

[9] McVay CS, Velásquez M, Fralick JA. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. Antimicrob Agents Chemother 2007; 51: 1934-8.

[10] Kumari S, Harjai K, Chhibber S. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by *Klebsiella pneumoniae* B5055. J Med Microbiol 2011; 60: 205-10.

[11] Abul-Hassan HS, El-Tahan k Massoud B, Gomaa R. Bacteriophage therapy of *Pseudomonas* burn wound sepsis. Annals of the MBC 1990; 3: 262-6. Available from: http://www.medbc.com/annals/review/vol_3/num_4/text/vol3n4p262.htm

[12] Soothill JS. Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa*. Burns 1994; 20: 209-11.

[13] Weber-Dabrowska B, Mulczyk M, Górski A. Bacteriophage therapy of bacterial infections: an update of our institute's experience. Arch Immunol Ther Exp (Warsz) 2000; 48: 547-51.

[14] Jikia D, Chkhaidze N, Imedashvili E, Mgaloblishvili I, Tsitlanadze G, Katsarava R, Glenn Morris J Jr, Sulakvelidze A. The use of a novel biodegradable preparation capable of the sustained release of bacteriophages and ciprofloxacin, in the complex treatment of multidrug-resistant *Staphylococcus aureus*-infected local radiation injuries caused by exposure to Sr90. Clin Exp Dermatol 2005; 30: 23-6.

[15] Markoishvili K, Tsitlanadze G, Katsarava R, Morris JG Jr, Sulakvelidze A. A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. Int J Dermatol 2002; 41: 453-8.

[16] Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. Burns 2006; 32: 644-6.

[17] Soothill J. Use of bacteriophages in the treatment of *Pseudomonas aeruginosa* infections. Expert Rev Anti Infect Ther 2013; 11: 909-15.

[18] Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP. European regulatory conundrum of phage therapy. Future Microbiol 2007; 2: 485-91.

[19] Huys I, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G. Paving a regulatory pathway for phage therapy. Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. EMBO Rep 2013; 14: 951-4.

[20] Verbeken G, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Huys I. Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp (Warsz) 2014; 62: 117-29.

[21] Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, Zizi M, Laire G, Lavigne R, Huys I, Van den Mooter G, Buckling A, Debarbieux L, Pouillot F, Azeredo J, Kutter E, Dublanchet A, Górski A, Adamia R. The phage therapy paradigm: prêt-à-porter or surmesure? Pharm Res 2011; 28: 934-7.

[22] Pirnay JP, Verbeken G, Rose T, Jennes S, Zizi M, Huys I, Lavigne R, Merabishvili M, Vaneechoutte M, Buckling A, De Vos D. Introducing yesterday's phage therapy in today's medicine. Future Virol 2012; 7: 379-90.

[23] Villareal LP. Overall issues of virus and host evolution. In: Villareal LP, editor. Viruses and the evolution of life. Washington DC: ASM Press; 2005. pp. 1-28.
[24] Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. Phage therapy in the food industry. Annu Rev Food Sci Technol 2014; 5: 327-49.
[25] Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 2009; 4: e4944.
[26] Berg JO, Mössner BK, Skov MN, Lauridsen J, Gottrup F, Kolmos HJ. Antibacterial properties of EMLA and lidocaine in wound tissue biopsies for culturing. Wound Repair Regen 2006; 14: 581-5.
[27] Perez-Cappellano R, Manelli JC, Palayret D, Carlin G, Echinard C, Jouglard JP. Evaluation of septicemia risk in burn patients. Parallel between skin bacterial count and blood culture. Nouv Presse Med 1976; 5: 1831-2.
[28] Taddonio TE, Thomson PD, Tait MJ, Prasad JK, Feller I. Rapid quantification of bacterial and fungal growth in burn wounds: biopsy homogenate Gram stain versus microbial culture results. Burns Incl Therm Inj 1988; 14: 180-4.

2.3.2 Bacteriophages for the treatment of severe infections: a ‘new’ option for the future?

D. De Vos, G. Verbeken, T. Rose, S. Jennes, J.P. Pirnay

EWMA Journal. 2012; 12(2):23-28

International scientific journal

Presented at
EWMA 2011
Brussels · Belgium

Bacteriophages for the treatment of severe infections: A 'new' option for the future?

INTRODUCTION

The worldwide emergence of "Superbugs" and a dry antibiotic pipeline threaten a return to the pre-antibiotic era, i.e. prior to the 1940s when millions of people died of bacterial infection[1].

In hospitals in both high-income and low-income countries, the majority of nosocomial outbreaks are caused by a small group of pathogens – *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa* and *Enterobacter* species, hereafter referred to as "the ESKAPE bugs."

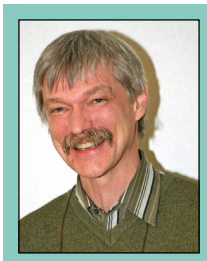
These ESKAPE bugs are increasingly prevalent in our hospitals and increasingly resistant to many of our antimicrobial agents threatening patients' lives and confronting society with huge socio-economic costs[1].

While extensively drug resistant *Acinetobacter baumannii*, often associated with military operations (Iraq, Afghanistan), NDM-1 containing *Enterobacteriaceae*, pan-resistant *Pseudomonas aeruginosa* clones and methicillin resistant *Staphylococcus aureus* (MRSA) are mainly prevalent in our hospitals, it seems that the community as a whole is threatened by these worrisome pathogens. This was demonstrated by the EAHEC 0104:H4 epidemic in Germany in 2011[2-5]. Some infectious agents are indeed not confined to human beings but actually deeply settled in our environment. Beside the overuse, and misuse of antibiotics in human medicine it seems also more and more evident that the animal food production sector serves as a major antibiotic consumer and consequently a reservoir for multi-drug resistant (MDR) bacteria. Our ever growing and crowded cities also seem to play a role in the emergence of these ESKAPE bugs[6-8]. Taking all this into account it is evident that the situation is alarming.

A reflection on the biological role of natural, as well as (semi-)synthetic antibiotics, in nature as secondary metabolites and their use as antimicrobial agents in human, veterinary and agro-bio industry reveals that we still have much to learn about these molecules. The lack of fundamental knowledge on the actual role of antibiotics (secondary metabolites often functioning as signalling molecules) in nature and their effect on living systems (bacteria) in relation with the whole ecological setting means that we actually disequilibrate our natural environment as a consequence of the mis/over use of those molecules. This biological phenomenon of antibiotic resistance is typically an emergent characteristic of a dynamic, highly complex and self-organizing system that evolves at the edge of chaos[9-10].

Antibiotics are typically studied and developed through models in which the bacteria are in a planktonic (free living and growing) life style, but most of the infections seem to be due to bacterial infectious foci, which mainly harbour bacteria that exhibit a biofilm life style[11]. It was shown by gene expression analysis that planktonic and biofilm lifestyle modes have distinct differential gene expression profiles. This affects, amongst other features, the bacterial sensitivity to antibiotics[12-13].

These bacterial biofilm-related findings imply that the mechanical barrier function of the biofilm is not the main reason why bacteria residing in a biofilm lifestyle mode do not respond as expected to antibiotics. Some antibiotics can diffuse into the biofilm complex and reach the bacteria, but as a consequence of the changed bacterial physiology and biochemical pathways in the biofilm modus some antibiotics cannot interfere with the biofilm bacteria in the same way as they would do with free living and proliferating planktonic bacteria. In a biofilm the bacterial growth rate is dramatically slowed down while the mechanical barrier protects them essentially from the immune system. Antibiotics were developed only taking into account the bacterium's planktonic lifestyle, but we know today that biofilms play a major role



[1]**Daniel De Vos**, PhD

[1]**Gilbert Verbeken**, MSc
[1,2]**Thomas Rose**, MD
[2]**Serge Jennes**, MD
[1]**Jean-Paul Pirnay**, PhD

[1]Laboratory for Molecular and Cellular Technology,

[2]Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium

Correspondence: daniel.devos2@mil.be

Conflict of interest: none

THE **EWMA** UNIVERSITY CONFERENCE MODEL (UCM)

in Vienna

EWMA 2012
23-25 May

The EWMA UCM programme offers students of wound management from institutes of higher education across Europe the opportunity to take part of their academic studies whilst participating in the EWMA Conference.

The opportunity of participating in the EWMA UCM is available to all teaching institutions with wound management courses for health professionals.

The UCM programme at the EWMA 2012 Conference in Vienna will focus on increasing the networking opportunities between the students from various UCM groups participating in the programme this year.

In addition to the main conference programme, UCM Lectures as well as assignments and workshops for mixed groups will be arranged specifically for the UCM students.

EWMA strongly encourages teaching institutions and students from all countries to benefit from the possibilities of international networking and access to lectures by many of the most experienced wound management experts in the world.

Yours sincerely

Zena Moore,
Chair of the Education Committee, Immediate Past President

Participating institutions:

Donau Universität Krems
Austria

Escola Superior de Enfermagem de Lisboa
Portugal

Haute École de Santé
Geneva, Switzerland

HUB Brussels
Belgium

KATHO university college Roeselare
Belgium

Lithuanian University of Health Sciences
Lithuania

University of Hertfordshire
United Kingdom

Universidade Católica Portuguesa
Porto, Portugal

For further information about the EWMA UCM, please visit the Education section of the EWMA website www.ewma.org or contact the EWMA Secretariat at ewma@ewma.org

in most infectious states[11]. New strategies, based on fundamental biofilm research to cope with this problem are under development. Recently, a review on bacterial biofilms was published in this journal by Antonio Fonseca[14].

Apart from the overuse and misuse of antibiotics there are thus several additional reasons for the antibiotic crisis which is partly a consequence of our current socio economic society[15]. The pharmaceutical industry is not eager to develop new antibiotics due to the long term resource-intensive research and development costs while knowing that eventually resistance will emerge and the return on investment will decrease. As the industry antibiotic pipeline is virtually dry and infectious diseases steadily on the increase, experts struggle to find acceptable solutions [16-18]. The use of bacteriophages, bacterio specific viruses, is currently being (re)considered as a sensible option. Last year several reports of clinical applications in animals and humans were published [19-24]. With our actual knowledge we can consider that bacteriophages are not harmful for eukaryotic organisms, such as humans. Eukaryotic organisms include fungi, plants and animals (including humans). They typically have a specific membrane-bound nucleus with its specific biochemical enzyme systems and organelles in contrast to the prokaryotic bacteria. Thus bacteriophages are bacterio-specific viruses that naturally cannot infect and replicate in a eukaryotic cell. In order to enter their host cell they need specific outer membrane receptors beside the specific bacterial biochemical machinery for replication. Bacteriophages (meaning bacteria eaters) are in fact the bacteria's natural predators. As such they keep bacterial populations growth under control. Wherever bacteria are present there are bacteriophages (or phages in short) which are generally present in at least a ten times higher order of magnitude than the bacteria themselves and consequently constitute the most abundant biological lifelike constituents of the biosphere of this planet[25]. This observation shows us that actually we live in an ocean of phages and have done since the dawn of the human species and that natural phages are in principle harmless to us. Ecologically they are key as bacterial controllers and it is this 'natural function' of bacteriophages that phage therapy is exploiting. In combination with or as substitute for antibiotics they could be a therapeutic option in the eradication or control of bacterial colonisations/infections. Indeed applying a specific natural lytic bacteriophage, targeted against a specific pathogenic bacterium, on for example an infected wound, should result in the lysis of the targeted bacterium after the amplification of the phage in the bacterial cell. As a result the wound would be cleared by the phage of its noxious bacteria. In fact the bacteriophage could be considered as a self am-

plifying drug at the place of infection. Once the bacteria are eradicated (through lysis) or brought to a low enough density that the host's immune system can take over the situation, the bacteriophages will also be eliminated by the host's immune system.

However today there is a lack of standardized evidence-based clinical research. This rediscovered antibacterial therapeutic approach, first proposed by d'Herelle almost a century ago, was only further developed, mostly empirically, in the former Soviet Empire [17-18, 26-29]. Since the early beginning of phage therapy this approach was continuously used in medical practice and empirically adapted so that today in countries like Georgia, phage therapy is considered an established medical practice not requiring any further questioning. To reintroduce it however in our actual medical practice requires clinical studies in accordance with current standards. But documenting a "lifelike" entity is not the same as documenting a chemical static substance, what an antibiotic in fact is. Also there is the aspect related to Intellectual Property Rights (IP) that after all looks to be the thorniest problem. Phage therapy could provide a sustainable solution for the multi-drug resistance crisis. Phage therapy is the use of natural exclusively lytic bacterio-specific viruses as antibacterial agent. In fact by setting up a screening system for the circulating noxious bacteria and their respective phages it will always be possible to obtain the right lytic phage against any emerging pathogen. This way of working, taking into account the co-evolution of the couplet bacterium/phage, makes it just a fitting solution for a sustainable antibacterial phage therapy industry. We think that phage therapy will surely have its (exclusive) application setting(s) and in addition could be used in combination (synergy) with antibiotics[30]. Studies show that phages can enhance antibiotic's activity by interaction with the bacterial biofilm modus. The search for a specific phage or phage cocktail against a specific bacterium will not take the time nor require the costs of searching and developing a new antibiotic. The search for a potent natural phage and the preparation of classic galenic preparation (physiological water, basic ointment…) containing phages is practical and feasible in the time frame of days to weeks, in contrast to new antibiotics which require many years of research and development. If an infection is caused by a pan-resistant bacterium it is realistic to select a specific phage for clinical use, in contrast to the search of a new antibiotic.

The clinical development of phage therapy however faces major obstacles, typical of the current medico-pharmaceutical environment, that hamper progress[18, 28-29, 31]

- The lack of a specific adapted regulatory frame in the medicinal product regulations (mainly based on the classic static chemical drugs)
- It is difficult to obtain IP, and as a consequence difficult to find investors
- The absence of well-defined, safe and targeted phage preparations (technically feasible, but due to the above mentioned reasons there are currently no dedicated therapeutic phage centres)
- The societal false perception of viruses as 'enemies of life'.

AIM AND METHOD

It was our aim to evaluate the potential of phage therapy and to bring it eventually to the patient.

A multidisciplinary team of biologists, medical doctors and pharmacists was established and worked simultaneously, from the start, on different aspects, ranging from the regulatory to the in vitro and in vivo (clinical) experiments of this antibacterial treatment.

- An exhaustive analysis of the current relevant drug or medicinal products regulatory frameworks was performed to analyse whether they could cater for phage therapy.
- A small-scale production process for the preparation of quality controlled and well-defined phage cocktails for clinical use was set-up. The elaboration of this project involved several research groups and a clinical team. Parts of the quality control tests would be outsourced. The final goal was to use this bacteriophage cocktail as a topical treatment against MDR *P. aeruginosa* and MRSA infected wounds in a pilot trial in burn wound patients with the agreement of a Belgian Medical Ethical Committee.
- To foster national and international interactions and to promote phage therapy in Europe, an international organization 'Phages for Human Applications Group – Europe' (P.H.A.G.E.) was created.

RESULTS AND DISCUSSION

An analysis of the regulatory framework and multiple discussions with several experts as well as the relevant competent authorities revealed that clinical phage therapy applications in the EU are possible, but that the regulatory frame is not well-adapted [28-29].

Although the development of phages as classical medicinal products like an antibiotics, including Good Manufacturing Practices (GMP) production, pre-clinical and phase I, II and III clinical trial and marketing is possible, it is, in our opinion, not the most appropriate route Such a developmental path would cost millions of Euros and take many years (± 10 years for biologicals). These investments are not compatible with the apparent lack of Intellectual Property (IP) protection (at least for natural

▶

phages). Phages, as natural entities, belong to mankind as a whole, and cannot be patented in the classical sense. Also the idea of phage therapy itself first put forward by Felix d'Herelle who coined the name of bacteriophages meaning 'bacterium eating entities', cannot, in principle, be patented since it belongs to the common knowledge and has done for almost a century. This situation does not stimulate the industry to invest, since the actual paradigm is "no IP, no investment"[15 -18].

To overcome this embarrassing situation, new views and consequent ways of (pharmaceutical) industrial models have to be developed[29].

Established pharmaceutical companies are not likely to invest substantial amounts of money and time in the development of potentially interesting products that will need to be adapted (evolve) even more quickly than flu vaccines, to be effective. This fast adaptation is needed to exploit the main advantage of phages over classical 'static' drugs such as antibiotics, namely their ability to rapidly (in a matter of days to weeks) evolve to target emerging pathogenic strains. This is possible by continuously screening bacteria and their phages, as is also done in Georgia. This "Sur-mesure" or tailor-made pathway for the future implementation of phage therapy is proposed and discussed by Pirnay *et al* [29]. This view is also what was proposed to the Innovation Task Force (ITF) at EMA. The discussion is still ongoing.

Non profit institutions like hospitals that would like to develop phage therapy are not necessarily disheartened by the IP issues and the uncertainty of large profits, but are generally unable to generate the necessary funding and are furthermore most likely better served by a tailor-made (e.g. to a patient or an outbreak) approach[29]. This means that in a timeframe of days to weeks a specific phage can always be found to target a specific emerging pathogen. It is this specific power of phage therapy, namely its co-evolutionary aspect, which guarantees an efficient antibacterial agent when needed.

As a result of this conundrum, until now, only local and sporadic phage applications were performed in the Western World, often under the umbrella of the Declaration of Helsinki. In Poland, an EU member state, a specific national adaptive regulation, based on the Declaration of Helsinki, was issued to regulate phage therapy. A medical doctor is allowed to apply phage therapy where proven therapeutic methods do not exist or have been ineffective (e.g. MDR infections) and provided that the patient or his legal representative signs an informed consent.

In France, Dr. Alain Dublanchet, a veteran of phage therapy, occasionally applies phages in desperate *osteomyelitis* cases and with success [24, 29] In Australia, phage therapy was recently applied under the umbrella of "compassionate use" for the successful treatment of refractory *P. aeruginosa* urinary tract infection in a cancer patient[24].

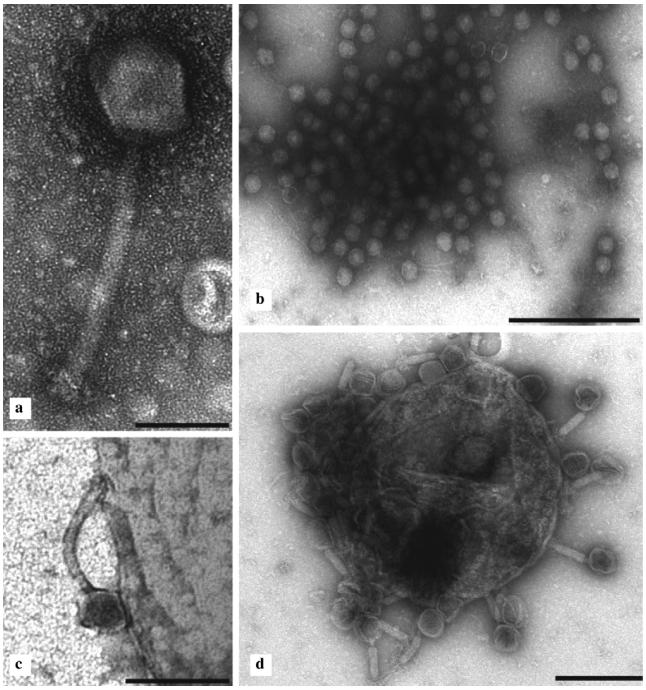


Figure 1.
BFC-1 transmission electron micrographs (.
a) P. aeruginosa bacteriophage 14/1, a member of the Myoviridae family. Bar: 100 nm.
b) PNM bacteriophages (Podoviridae) freed from a burst P. aeruginosa bacterium. Bar: 500 nm.
c) Bacteriophage 14/1 attaching to the P. aeruginosa cell wall. Bar: 200 nm.
d) ISP bacteriophages (Myoviridae) attaching to S. aureus. Bar: 500 nm.
Ref. 26 Merabishvili et al 2009.

In Belgium a basic clinical safety trial was performed with the approval of a leading Medical Ethical Committee.

Clinical trials of course need safe and well-defined phages. Therefore a phage cocktail (BFC-1) that targeted the most prevalent MDR *P. aeruginosa* and MRSA bacteria was produced. The cocktail consisted of two phages against *P. aeruginosa* and one against *S. aureus* (Fig. 1). It was produced on a small scale and in accordance with basic clinical-pharmaceutical standards (sterility, apyrogenicity, pH, cytotoxicity, adequate shelf life and stability). In addition, the phages in BFC-1 were proven to be exclusively lytic and characterized at the genomic and proteomic level. This specific production process was published by Merabishvili *et al.*[32] and is actually used as a basic discussion document for future adaptations in the regulatory documentation process.

BFC-1 was applied, in a small pilot study, in the burn unit of the Queen Astrid Military Hospital (9 patients, 10 applications) (Fig. 2). This was one of the first unconcealed phage applications in modern Western medicine. As expected, no adverse events or side effects were observed based on clinical as well as laboratory-measurable param-

Figure 2.a.
The final product, a bacteriophage cocktail ready for use in a human clinical trial. (Ref 26)

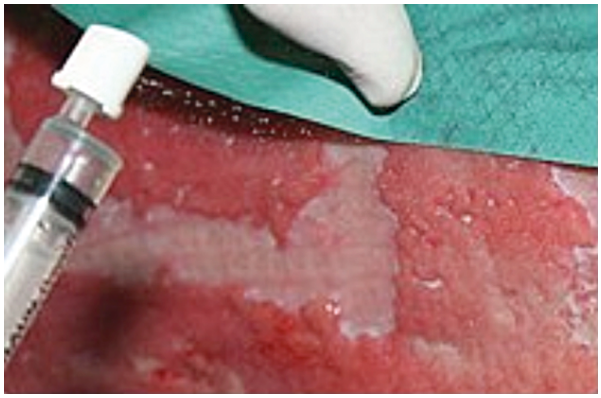


Figure 2.b.
Application of BFC-1 on an infected burn wound using a syringe spray. (Ref 26)
Actually phages could be applied by a spray or a galenic ointment formulation. Before application on wounds the wound bed should always be cleaned, debrided and rinsed with bicarbonated physiological water in order to provide a neutral pH environment. This is to allow the phages to be stable. Too acidic or alkaline environments cause phage degradation (protein denaturation). Studies are warranted in order to optimize applications and frequency of application as well as the type of the most suited galenic formulation in function for the site of use.

eters. This small pilot safety trial, showing the innocuity of phages when applied to burns, was discussed in a review by Kutter and colleagues[27]. In addition, we successfully applied (systemically, through a wound drain) large quantities of BFC-1 (300 ml of 10^5 phage particles), under the Declaration of Helsinki, in a critical pelvic trauma patient with MDR *P. aeruginosa* and MRSA osteomyelitis.

Over the years, it has become clear that, in order to develop phage therapy, an adapted regulatory framework and eventually even a change in (medical/pharmaceutical) mentality and developmental models needs to be achieved. Especially the natural evolutionary and sustainability aspects of the approach, not compatible with our current bio/pharmaceutical business models where IP issues are at the core, have to be taken into consideration when developing phage therapy. The P.H.A.G.E. network allowed us to discuss fundamental and practical issues such as the status of phages (e.g. are they (classical) drugs?), exchange information on applications and services and subsequently to efficiently interact with authorities like the European Medicines Agency (EMA).

In February 2011 we officially interpellated the EU parliament: 'what is the status of phage as antibacterial agent' which brought the discussion to the European level. The question was put on the agenda by the Belgian Christian democrat Ivo Belet and his colleague Catherine Trautmann from the Socialist faction in France. The Commission's view was that the current regulatory framework was sufficient for "phage therapy", a standpoint we clearly don't share. Indeed if we consider the phage as a static chemical substance we cannot develop phage therapy as it should be developed in a sustainable efficacious way and tailor made as discussed by Pirnay *et al*[29].

Concerning the "false perception of viruses as enemies of life" obstacle, which we feared when starting our clinical trial, we found – to our surprise – that it was easily resolved through clear and scientific communication with the members of the ethical committee as well as the medical and nursing staff of the hospital.

CONCLUSION

Natural phages are not straightforward inanimate and stable substances, but rather lifelike evolvable natural biological entities. The major obstacle hampering the further development of phage therapy at large, in wound treatment as well as in other clinical settings (otitis, osteomyelitis, diabetic foot, diarrhoea, impetigo…) in our current medical/pharmaceutical environment is mainly related to the intellectual IP issues.

The existing relevant regulatory frameworks and business models are not compatible with a dynamic sustainable phage therapy concept. And this point of view is not compatible with the current economic models that reduce the pharmaceutical industry to 'common button' producers, when their main societal role should be 'providing people with adequate products for a better health'. Therefore a suitable environment should be worked out [28-29]. We need to radically redesign our (pharmaceutical) economic models to cater for more dynamic and sustainable approaches that fit an eventual future green economy. We are actually bouncing against our own 'limits' of growth [33-34].

Any future sustainable phage therapy concept should, based on scientific grounds, fully acknowledge the potentialities of the co-evolutionary aspect of the couplet phage/bacterium in its ecological environment, in casu the human being[29]. Only then the inherent (positive) characteristics of phages as natural biological bacterium controllers can be put to use. Indeed, bacteria will inevitably ▶

become resistant to phages, but due to the continuously ongoing arms race between the two protagonists, specific phages able to infect the formerly resistant bacterial strains will quickly emerge[29]. In fact phage therapy fits well in the new emerging field of Darwinian – evolutionary – medicine (in contrast to a classical mechanistic – man as a machine – view) where the insights of evolution are fully taken into account. Viruses, among which phages are included, were involved in the origin of life itself and play a major role in biological evolution [35-36]. Hopefully they will play a role in the future control of bacterial disease. We feel that our plea for a more realistic approach, taking into account the co-evolutionary aspect of the bacterium and its phage is scientifically sound. Let's hope that the political and economic factors will adapt.

References

1. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis 2008: 197(8): 1079-81.
2. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 2004: 10 (12): S122-9.
3. Kumarasamy K, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010: 10(9): 597-602.
4. Brzuskiewicz E, Thurmer A, Schuldes J, Leinbach A, Lieregang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathogen type Entero-Aggregative-Haemorrhagic-Escherichia coli (EAHEC). Arch Microbial 2011: 193: Epub ahead of print.
5. Pirnay JP, De Vos D, Cochez C, Bilocq F, Pirson J, Struelens M, Duinslaeger L, Cornelis P, Zizi M, Vanderkelen A. Molecular epidemiology of *Pseudomonas aeruginosa* colonization in a burn unit: persistence of a multidrug-resistant clone and a silver sulfadiazine-resistant clone. J Clin Microbiol 2003: 41(3): 1192-202.
6. Kümmerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin Microbiol Infect 2003: 9(12):1203-14.
7. Kümmerer K. Resistance in the environment. J Antimicrob Chemother 2004: 54(2): 311-20.
8. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010: 8(4): 251-259.
9. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev 2002: 15(4): 647-79.
10. Baquero F, Coque TM, Canton R. Antibiotics, complexity and evolution. ASM News. 2003: 69(11): 547-52.
11. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999: 284(5418): 1318-22.
12. Whiteley M, Bangera MG, Burngarner RE, Parsek MR, Teitzel GM, Lorry S, Greenberg EP. Gene expression in *Pseudomonas aeruginosa* biofilms. Nature 2001: 413(6858): 860-4.
13. Beloin C, Valle J, Latour-Lambert P, Faure P, Kzreminski M, Balestrino D, Haagensen JAJ, Molin S, Prensier G, Arbeille B, Ghigo JM. Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. Mol Microbiol 2004: 51(3): 659-74.
14. Fonseca AP. Biofilms in wounds: an unsolved problem? EWMA Journal. 2011: 11(2): 10-23.
15. Pirnay JP, De Vos D , Zizi M , Heyman P. No easy way to exterminate 'superbugs' at the dawn of the third millennium. Expert Rev Anti Infect Ther 2003: 1(4): 523-25.
16. Bush K , Courvalin P , Dantas G , Davies J , Eisentstein B , Huovinen P , Jacoby GA , Kishony R , Kreiswirth BN , Kutter E , Lerner SA , Levy S , Lewis K , Lomovskaya O , Miller, JH , Mobashery S , Piddock LJV , Projan S , Thomas CM , Tomasz A , Tulkens PM , Walsh TR , Watson JD , Witkowski J , Witte W , Wright G , Yeh P, Zgurskaya HI. Tackling antibiotic resistance. Nat Rev Microbiol 2011: 9(11): 894-96.
17. Maura D, Debarbieux L. Bacteriophages as twenty-first century antibacterial tools for food and medicine. Appl Microbiol Biotechnol 2011: 90(3): 851-9.
18. Thiel K. Old dogma, new tricks-21st. century phage therapy. Nature Biotechnol 2004: 22(1): 31-6.
19. Markoishvili K, Tsitlanatze G, Katsarava R, Morris JG Jr, Sulakvelidze A. A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. Int J Dermatol. 2002: 41(7): 453-8.
20. Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. Burns 2006: 32(5):644-6.
21. McVay CS, Velasquez M, Fralick JA. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. Antimicrob Agents Chemother. 2007: 51(6): 1934-8.
22. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care. 2009: 18(6):237-43.
23. Kumari S, Hariai K, Chibber S. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by *Klebsiella pneumoniae* B5055. J Med Microbiol. 2011: 60(Pt2): 205-10.
24. Khawalkaled A, Morales S, Dillon B, Alavidze Z, Ginn AN, Thomas L, Chapman SJ, Dublanchet A, Smithyman A, Iredell JR. Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. J Med Microbiol 2011: 60(11): 1697-700.
25. Hendrix RW. Bacteriophages: evolution of the majority. Theor Popul Biol 2002: 61(4): 471-80.
26. D'Herelle F. Sur un microbe invisible antagoniste des bactéries dysentériques. C R Acad Sci 1917: 165: 373-5.
27. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 2010: 11(1): 69-86.
28. Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP. European regulatory conundrum of phage therapy. Future Microbiol 2007: 2(5): 485-91.
29. Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, Zizi M, Laire G, Lavigne R, Huys I, Van den Mooter G, Buckling A, Debarbieux L, Pouillot F, Azeredo J, Kutter E, Dublanchet A, Gorski A, Adamia R. The Phage paradigm: prêt-à-porter or sur-mesure? Pharm Res 2010: 28(4): 934-7.
30. Comeau AM, Tétart F, Trojet SN, Prère MF, Krish HM. Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phagegrowth. PLoS One 2007: 2(8): e799.
31. Villarreal LP. Overall issues of viruses and host evolution. In: Villareal LP, ed. Viruses and the Evolution of Life. W. A., USA: ASM press; 2005 p. 1-28.
32. Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashki N, Gionti T, Krylov V, Mast J, Van Parijs L, Lavigne R, Volckaert G, Matheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One. 2009: 4: e4944.
33. The Group of Lisbon. Limits to Competition. Cambridge, Mass., USA: The MIT Press; 1995.
34. Sachs JD. Common Wealth: economics for a crowded planet. London, UK: Penguin, 2008.
35. Buckling A, Rainey PB. Antagonistic coevolution between a bacterium and a bacteriophage. Proc Biol Sci 2002: 269(1494): 931-6.
36. Villarreal LP, Witzany G. Viruses are essential agents within the roots and stem of the tree of life. J Theor Biol 2010: 262(4): 698-710.

3 Development of a bacteriophage therapy concept

3.1 Regulatory concept design (Study 4)

3.1 Regulatory concept design (Study 4)

3.1.1 The bacteriophage therapy paradigm: *Prêt-à-Porter* or *Sur-Mesure*

J.P. Pirnay, D. De Vos, G. Verbeken, M. Merabshvili, N. Chanishvili, M. Vaneechoutte, M. Zizi, G. Laire, R. Lavigne, I. Huys, G. Van den Mooter, A . Buckling, L. Debarbieux, F. Pouillot, J. Azerado, E. Kutter, A. Dublanchet, A. Górski, R. Adamia

Pharm Res. 2010; DOI 10.1007/sII095-010-0313-5

International scientific journal, peer-reviewed

3.1.2 Introducing yesterday's bacteriophage therapy in today's medicine

J.P. Pirnay, G. Verbeken, T. Rose, S. Jennes, M. Zizi, I. Huys, R. Lavigne, M. Merabishvili, M. Vaneechoutte, A. Buckling, D. De Vos

Future Virol. 2012; 7(4):379-390

International scientific journal, peer-reviewed

3.1.3 Paving a regulatory pathway for bacteriophage therapy

I. Huys, J.P. Pirnay, R. Lavigne, S. Jennes, D. De Vos, M. Casteels, G. Verbeken

Embo reports. 2013; 14(11):951-954

International scientific journal

3.1.1 The bacteriophage therapy paradigm: *Prêt-à-Porter* or *Sur-Mesure*

J.P. Pirnay, D. De Vos, G. Verbeken, M. Merabshvili, N. Chanishvili, M. Vaneechoutte, M. Zizi, G. Laire, R. Lavigne, I. Huys, G. Van den Mooter, A . Buckling, L. Debarbieux, F. Pouillot, J. Azerado, E. Kutter, A. Dublanchet, A. Górski, R. Adamia

Pharm Res. 2010; DOI 10.1007/sIl095-010-0313-5

International scientific journal, peer-reviewed

Pharm Res
DOI 10.1007/s11095-010-0313-5

COMMENTARY

The Phage Therapy Paradigm: *Prêt-à-Porter* or *Sur-mesure*?

Jean-Paul Pirnay • Daniel De Vos • Gilbert Verbeken • Maia Merabishvili • Nina Chanishvili • Mario Vaneechoutte • Martin Zizi • Geert Laire • Rob Lavigne • Isabelle Huys • Guy Van den Mooter • Angus Buckling • Laurent Debarbieux • Flavie Pouillot • Joana Azeredo • Elisabeth Kutter • Alain Dublanchet • Andrzej Górski • Revaz Adamia

Received: 22 July 2010 / Accepted: 27 October 2010

KEY WORDS antibiotic resistance · bacterial infection · bacteriophages · drugs · phage therapy

The present opinion is the result of discussions on the future of phage therapy (personalized or large-scale uniform therapy?) during the first International Congress on Viruses of Microbes, held at the Institut Pasteur in Paris on June 21–25, 2010.

Antibiotics are becoming ineffective as important bacterial pathogens evolve to outsmart them. Yet the antibiotic pipeline is running dry with only a few new antibacterial drugs expected to make it to the market in the foreseeable future. Bacteria that are resistant to all available antibacterial drugs, so-called *superbugs*, are emerging worldwide. Evolutionary ecology might inform practical attempts to bring these pathogens under stronger human control (1).

In this context, various laboratories worldwide and a handful of small pharmaceutical companies are turning to (bacterio)phages (2). Phages are natural viruses that specifically infect bacteria. They are (among) the most abundant and ubiquitous lifelike entities on Earth and coevolve with their hosts, the bacteria. Lytic phages bind to receptors on the bacterial cell surface, inject their genetic material, use the bacterium's reproductive machinery to replicate and subsequently destroy (lyse) the bacterium, irrespective of its resistance to antibiotics, releasing the newly formed phages to seek out new hosts.

In 1919, d'Herelle used phages to treat dysentery in Paris, in what was probably the first attempt to use phages therapeutically. d'Herelle eventually developed a commercial laboratory in Paris that produced phage preparations against

J.-P. Pirnay · D. De Vos · G. Verbeken · M. Merabishvili
Laboratory for Molecular and Cellular Technology
Burn Wound Centre, Queen Astrid Military Hospital
1120 Brussels, Belgium

M. Merabishvili · N. Chanishvili · R. Adamia
Eliava Institute of Bacteriophage Microbiology and Virology
380060 Tbilisi, Georgia

M. Merabishvili · M. Vaneechoutte
Laboratory of Bacteriology Research, Faculty of Medicine
Ghent University
9000 Ghent, Belgium

M. Zizi
Department of Physiology Free University Brussels
1090 Brussels, Belgium

M. Zizi
Section Health of the Division Well-Being (Belgian Defence Staff)
Queen Astrid Military Hospital
1120 Brussels, Belgium

G. Laire
Office of the Surgeon General, Belgian Defence
1140 Evere, Belgium

R. Lavigne
Laboratory of Gene Technology, Katholieke Universiteit Leuven
3001 Leuven, Belgium

I. Huys
Faculty of Law, Centre for Intellectual Rights
Catholic University of Leuven
3000 Leuven, Belgium

G. Van den Mooter (✉)
Laboratory of Pharmacotechnology and Biopharmacy
Catholic University of Leuven
Herestraat 49
3000 Leuven, Belgium
e-mail: guy.vandenmooter@pharm.kuleuven.be

Published online: 10 November 2010

various bacterial infections, which were marketed by what later became the large French company L'Oréal (3). In the 1930s, therapeutic phages were also marketed in the United States by major pharmaceutical companies including Eli Lilly, Squibb & Sons (today Bristol-Meyers Squibb) and the Swan-Meyers division of Abbott Laboratories. Scientific controversies and the advent of antibiotics, however, relegated phage therapy to complete obscurity in most of the Western world. Nevertheless, phage therapy was further developed and extensively used in Eastern Europe and the former Soviet Union with activities centered at the Eliava Institute of Bacteriophage, Microbiology, and Virology (EIBMV) in Tbilisi, Georgia, several institutes in Russia, and the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland.

Despite its long (Eastern European) history, phage therapy is not currently authorized for routine use on humans in the West. Today, it is only approved in some former Soviet republics like Russia and Georgia, where commercial phage preparations are sold in pharmacies (4). In Poland, a recent member of the European Union, phage therapy is considered an 'Experimental Treatment' covered by the Physician Practice Act (Polish Law Gazette N° 28 of 1997) and the declaration of Helsinki, where other therapeutic options do not exist (5). In France, therapeutic made-to-order phage preparations from the Institut Pasteur (Paris and Lyon) were used until the beginning of the nineties. Today, a French practitioner, Alain Dublanchet, still uses commercial phage preparations (purchased in Russia and Georgia) to treat severe infections. Despite the absence of a specific framework for phage therapy (6), a pilot clinical trial in burn wounds was approved by a leading ethical committee in Belgium (7). In the United States, a Food and Drug Administration (FDA)-approved phase I clinical trial was conducted. No safety concerns were found (7). Recently, a British phage therapy company conducted a phase I/IIa clinical trial in chronic otitis. This study was approved through the UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review process (7).

Phages are harmless to eukaryotic (*e.g.* animal or plant) cells and are reported to elicit few, if any, side effects in humans. In contrast to antibiotics, they target specific bacterial species or even strains and can thus be chosen to be harmless for the non-target beneficial commensal flora (*e.g.* the gut flora) of the patient. This specificity also means that the right match between the phages and the targeted bacterial pathogen must be found. To improve the chance of success, off-the-shelf phage preparations should contain multiple phage strains per targeted bacterial species. This phage mixture should target the bacterial strains that are most commonly present at the intended point of use.

As with antibiotics, bacteria can evolve resistance to phages during the course of treatment (*e.g.* by alteration of phage receptors), to survive the phage attacks. This might result from mutations acquired during the course of treatment, but it is also likely that resistant bacteria are already present in the target population before phage treatment. Indeed, it is not in phages' best interest to kill all the host bacteria in the infection site, but they can be expected to (bio)control the bacterial pathogens and significantly reduce their numbers and thus give the patient's immune system and/or antibiotics the chance to eliminate the remaining bacteria. Moreover, *in vitro*, natural selection drives the rapid emergence of new phages that can destroy bacteria that have become resistant (9), and this may also be important in clinical contexts.

Ninety years of phage therapy have shown that after a while phage preparations become less effective and need to be updated. The ineffective phages can either be "trained," a term used in the EIBMV to indicate the selection of phage mutants more active against the phage-resistant bacteria, or replaced by new active phages. New phages are generally selected from the environment (*e.g.* sewage water), but in some cases they can be isolated from clinical

A. Buckling
Department of Zoology, University of Oxford
Oxford OX1 3PS, UK

L. Debarbieux
Department of Microbiology
Molecular Biology of the Gene in Extremophiles Unit
Institut Pasteur
75724 Paris, France

F. Pouillot
Pherecydes Pharma
93230 Romainville, France

J. Azeredo
Institute for Biotechnology & Bioengineering
Centre of Biological Engineering
Universidade do Minho
Campus de Gualtar
4710-057 Braga, Portugal

E. Kutter
Phage Biology Lab, The Evergreen State College
Olympia, Washington 98505, USA

A. Dublanchet
Laboratoire de Microbiologie, Centre Hospitalier Intercommunal de Villeneuve-Saint-Georges
94195 Villeneuve Saint-Georges, France

A. Górski
Transplantation Institute, The Medical University of Warsaw Institute of Immunology and Experimental Therapy
Polish Academy of Sciences Wroclaw
Wroclaw, Poland

samples containing the problematic bacterium. In phage therapy centers in Georgia and Poland, banks containing many different phages are kept and regularly updated. Sometimes custom phage preparations are developed for a patient's infection (autophage), a procedure that usually takes a few days to weeks.

This *sur-mesure* approach is not compatible with the current licensing processes. Recently, the European Medicines Agency (EMA) placed phages under the Medicinal Product Regulation and more specifically under the category of biologicals. Also, in the US, the amount of research and testing required by the FDA is seriously hampering the resurgence of phage therapy. Regulators impose many years of research and clinical trials, which cost millions of euros, to entrepreneurs to develop and distribute phage preparations (Fig. 1).

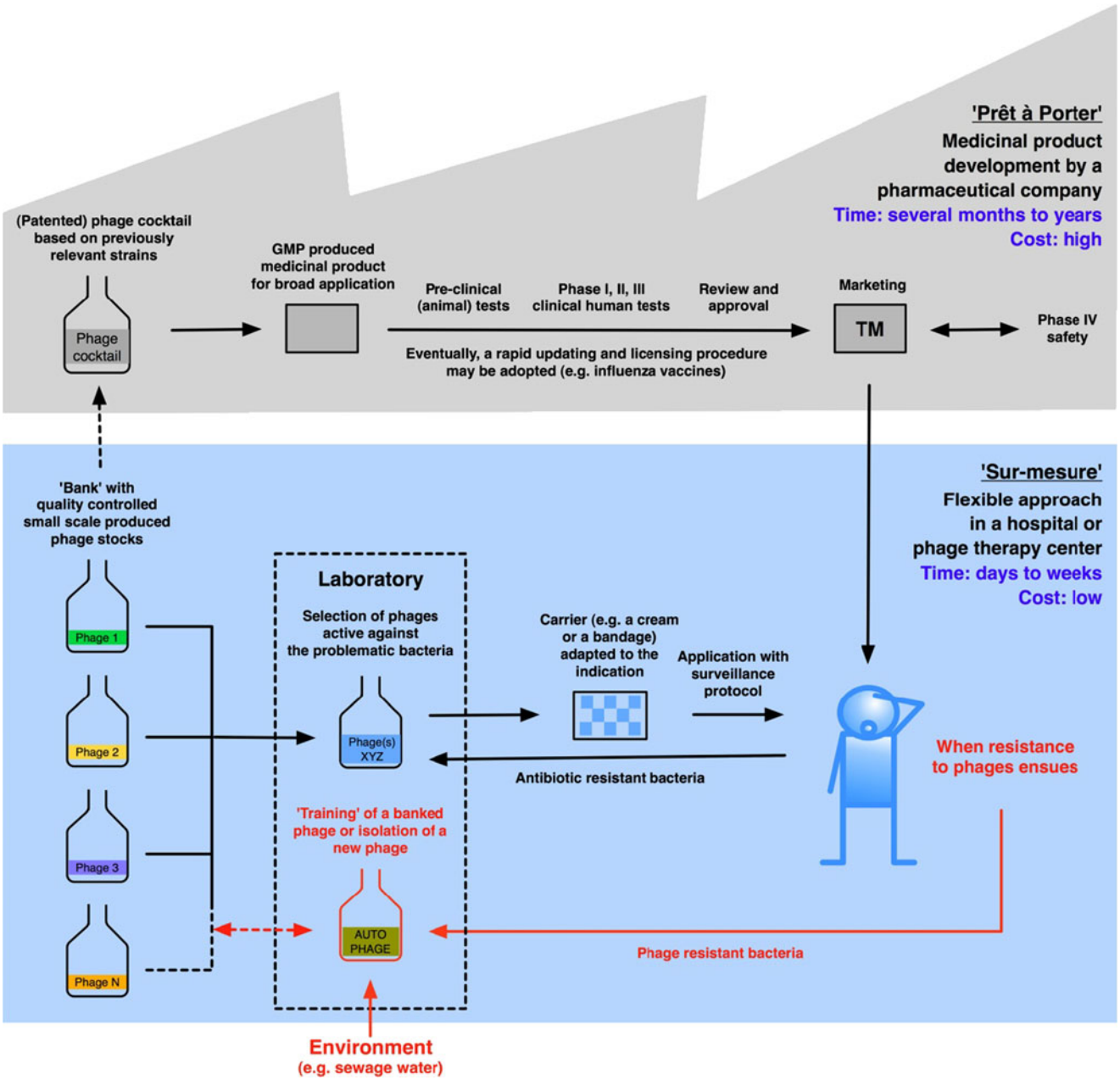


Fig. 1 Phage therapy concepts: *prêt-à-porter* or *sur-mesure?* Both approaches should be possible and could even be complementary. The specificity of phages, resistance and IP issues may thus hamstring pharmaceutical companies in the worldwide marketing of generic phage preparations. The long and expensive regulatory pathways, on the other hand, form insurmountable obstacles for *bonafide* or non-profit phage therapy centers or hospitals, which opt for a *sur-mesure* concept, and for institutions that would like to use inexpensive phages for commercially unattractive applications, in emerging countries, for example. Of course, this *sur-mesure* approach should also adhere to certain standards of behavior, safety and quality control (8). These standards could be defined in a new and specific section for phage therapy under the Advanced Therapy Medicinal Product Regulation (6).

Notwithstanding these regulatory hurdles and the empirical evidence suggesting that stable and widely distributed phage preparations (*prêt-à-porter*) will only be of (time-)limited use, a few companies have picked up the gauntlet and are moving along the elaborate and expensive licensing pathways. If nothing else, these efforts will put phage therapy back on the map in the Western world, and, once commonly accepted, EMA and FDA might revise their rules the way they did for influenza vaccines, which also require a rapid updating and licensing procedure (10). However, are pharmaceutical companies willing to commit to rapidly and regularly adapting their phage preparations to very specific or newly emerging demands? Take, for example, a hospital unit confronted with an MDR bacterial strain that causes untreatable infections in only one or two patients. Phage therapy is probably best—but not exclusively—served by small-scale productions and distributions of locally adapted or personalized phage preparations (cottage industry) (Fig. 1).

To avoid the drug licensing pathway, some US-based phage companies decided to first develop phage products for the decontamination of food, plants, fields and livestock (2). They hope to create revenue to fund research into human therapeutics and to familiarize the authorities and the general public with phages. Phages for decontaminating food plants, ready-to-eat meat, poultry products, cheese and live animals that will be slaughtered for human consumption were approved by the FDA and are now in use. Very little is known, however, about the impact of such massive and widespread applications of phages on natural microbial communities.

The lack of strong intellectual property (IP) protection is another discouraging factor for pharmaceutical companies. The principle of phage therapy has been common knowledge since the 1920s, and many aspects might thus be unpatentable. In addition, there are indications that in the future, phages, which are natural entities composed of genetic material and proteins, will only be patentable if they have been engineered into something distinctly different in character (11). Engineered phages could be patented, but, considering the current concerns about potential risks for public health and the environment which may arise from genetic engineering in genetically modified organisms (GMOs), they are not likely to be given licensing approval in the near future. Phage-derived products (*e.g.* cell wall-degrading enzymes such as endolysins) can and probably will be licensed and marketed within a few years. They may also select resistance, but presumably at a lower rate than antibiotics. Of course, these phage products lack the capacity of self-replication and adaptation in the infectious site.

Phage therapy has great potential in some (niche) clinical contexts, but as with antibiotic treatment, there are likely to be important evolutionary consequences (12) if it is implemented widely and without sufficient oversight. Some aspects of phage-bacterium evolution ecology (e.g. emergence of resistance) should first be analyzed in the light of future phage therapy. Real-time experimental evolution studies could help determine these evolutionary consequences and generate the analytical knowledge in support of the empirical knowledge and clinical experience that was accumulated in the Eastern world. More importantly, they will hopefully enable the creation of a rational phage therapy concept (Fig. 1), thus avoiding the historical mistakes that occurred in the course of antibiotic therapy development and which lead to the current massive and widespread occurrence of antibiotic resistance in the patient population as well as in the natural environment.

REFERENCES

1. Williams PD. Darwinian interventions: taming pathogens through evolutionary ecology. Trends Parasitol. 2010;26:83–92.
2. Thiel K. Old dogma, new tricks—21st Century phage therapy. Nat Biotechnol. 2004;22:31–6.
3. Sulakvelidze A, Alavidze Z, Morris Jr JG. Bacteriophage therapy. Antimicrob Agents Chemother. 2001;45:649–59.
4. Chanishvili N, Sharp R. Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia. A literature review of the practical application of bacteriophage research. Tbilisi: Eliava Foundation; 2009.
5. Górski A, Miedzybrodzki R, Borysowski J, Weber-Dabrowska B, Lobocka M, Fortuna W, *et al.* Bacteriophage therapy for the treatment of infections. Curr Opin Investig Drugs. 2009;10:766–74.
6. Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay J-P. European regulatory conundrum of phage therapy. Future Microbiol. 2007;2:485–91.
7. Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, *et al.* Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol. 2010;11:69–86.
8. Merabishvili M, Pirnay J-P, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, *et al.* Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS ONE. 2009;4:e4944.
9. Buckling A, Rainey PB. Antagonistic coevolution between a bacterium and a bacteriophage. Proc Biol Sci. 2002;269:931–6.
10. Wood JM, Levandowski RA. The influenza vaccine licensing process. Vaccine. 2003;21:1786–8.
11. Akst J. Key cancer patents killed. http://www.the-scientist.com/blog/display/57265/ (accessed 19/10/10), part of The Scientist. http://www.the-scientist.com/ (accessed on 19/10/10).
12. Levin BR, Bull JJ. Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol. 2004;2:166–73.

3.1.2 Introducing yesterday's bacteriophage therapy in today's medicine

J.P. Pirnay, G. Verbeken, T. Rose, S. Jennes, M. Zizi, I. Huys, R. Lavigne, M. Merabishvili, M. Vaneechoutte, A. Buckling, D. De Vos

Future Virol. 2012; 7(4):379-390

International scientific journal, peer-reviewed

Review
Future Virology

Introducing yesterday's phage therapy in today's medicine

Jean-Paul Pirnay*[1], Gilbert Verbeken[1], Thomas Rose[1], Serge Jennes[2], Martin Zizi[3,4], Isabelle Huys[5,6], Rob Lavigne[7], Maia Merabishvili[1,8,9], Mario Vaneechoutte[8], Angus Buckling[10] & Daniel De Vos[1]

[1]Laboratory for Molecular & Cellular Technology, Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium
[2]Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium
[3]Well Being Department, Queen Astrid Military Hospital, Brussels, Belgium
[4]Department of Physiology, Free University Brussels, Brussels, Belgium
[5]Department of Pharmaceutical & Pharmacological Sciences, Centre for Pharmaceutical Care & Pharmacoeconomics, KU Leuven, Leuven, Belgium
[6]Center for Intellectual Property Rights, KU Leuven, Leuven, Belgium
[7]Laboratory of Gene Technology, KU Leuven, Leuven, Belgium
[8]Laboratory of Bacteriology Research, Faculty of Medicine & Health Sciences, Ghent University, Ghent, Belgium
[9]Eliava Institute of Bacteriophage, Microbiology, & Virology, Tbilisi, Georgia
[10]Biosciences, University of Exeter, Cornwall Campus, Penryn, UK
*Author for correspondence: Tel.: +32 2 264 4844 ■ Fax: +32 2 264 4907 ■ jean-paul.pirnay@mil.be

The worldwide emergence of 'superbugs' and a dry antibiotic pipeline threaten modern society with a return to the preantibiotic era. Phages – the viruses of bacteria – could help fight antibiotic-resistant bacteria. Phage therapy was first attempted in 1919 by Felix d'Herelle and was commercially developed in the 1930s before being replaced by antibiotics in most of the western world. The current antibiotic crisis fueled a worldwide renaissance of phage therapy. The inherent potential of phages as natural biological bacterium controllers can only be put to use if the potential of the coevolutionary aspect of the couplet phage–bacterium is fully acknowledged and understood, including potential negative consequences. We must learn from past mistakes and set up credible studies to gather the urgently required data with regard to the efficacy of phage therapy and the evolutionary consequences of its (unlimited) use. Unfortunately, our current pharmaceutical economic model, implying costly and time-consuming medicinal product development and marketing, and requiring strong intellectual property protection, is not compatible with traditional sustainable phage therapy. A specific framework with realistic production and documentation requirements, which allows a timely (rapid) supply of safe, tailor-made, natural bacteriophages to patients, should be developed. Ultimately, economic models should be radically reshaped to cater for more sustainable approaches such as phage therapy. This is one of the biggest challenges faced by modern medicine and society as a whole.

Spreading antibiotic resistance: a universal threat

The worldwide emergence of 'superbugs' and a dry antibiotic pipeline threaten modern society with a return to the preantibiotic era, when bacterial infections were the primary cause of morbidity and mortality [1]. A recent estimate indicates that 400,000 people in Europe were infected with multidrug-resistant (MDR) bacteria during 2007, with 25,000 attributable deaths [2]. In hospitals in both the developed and the developing world, the majority of nosocomial outbreaks are caused by a small group of pathogens (i.e., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, hereafter referred to as the 'ESKAPE bugs') [3]. These ESKAPE bugs are increasingly prevalent and resistant to most of our antimicrobial agents, threatening patients' lives and confronting society with huge socioeconomic costs. To date, MDR pathogens, such as highly drug-resistant *A. baumannii* (often associated with military operations in the Middle East [4]), NDM-1-producing *Enterobacteriaceae* [5], pan-resistant *P. aeruginosa* clones [6] and methicillin-resistant *S. aureus* (MRSA) [7], have been mostly associated with hospital outbreaks.

In addition, community-associated MRSA infections and specific *Escherichia coli* outbreaks demonstrate that the community as a whole is increasingly threatened by virulent antibiotic-resistant pathogens. Community-associated MRSA infections arise in otherwise healthy individuals and are more virulent and transmissible than are traditional

Keywords
■ antibiotic ■ bacteriophage ■ bacterium ■ coevolution ■ drug ■ infection ■ medicinal product ■ multidrug-resistant ■ patent ■ phage ■ resistance ■ therapy

Future Medicine part of fsg

hospital-associated MRSA strains [8], and the recent outbreak of enteroaggregative Shiga toxin/verotoxin-producing *E. coli* strain O104:H4 in Germany [9] caused over 4000 cases of diarrhea – 3167 without hemolytic–uremic syndrome (16 deaths) and 908 with hemolytic–uremic syndrome (34 deaths) [10]. These cases demonstrate that infectious agents are not confined to hospitalized patients, but are actually deeply settled in our environment.

For rapidly evolving, genetically versatile bacteria such as *Pseudomonas*, it has turned out to be quite easy to develop mechanisms to avoid the toxicity of antibiotics, which have remained more or less 'static' for the last decade. More reflection on the biological role of antibiotics in nature as secondary metabolites would have revealed that resistance evolution was inevitable. Also, in nature, bacteria are constantly outsmarting toxins produced by competitors. However, the difference is that these natural competitors in turn react by selection towards adjusted toxins. The biological phenomenon of antibiotic resistance is typically an emergent characteristic of a dynamic, highly complex and self-organizing system that evolves at the edge of chaos [11,12]. Moreover, the rate of resistance evolution has been exacerbated by the overuse and misuse of antimicrobial agents in both clinical and agricultural contexts [13–15].

Due to the complexity of the antibiotic resistance issue and the immense research and development costs and time-frames of developing new antibiotics, for which resistance will inevitably occur, the pharmaceutical industry is not keen to continue with the development of new molecules. Moreover, even if pharmaceutical companies succeed in developing and marketing highly active antibiotics, authorities, sensitized by past experiences concerning the rapid emergence of resistance, are likely to withhold these new antibiotics as third-line last-rescue drugs, thereby limiting the market and consequently the commercial interest of the pharmaceutical companies. As the industry antibiotic pipeline is virtually dry and infectious diseases – major causes of morbidity and mortality – are steadily on the increase, new initiatives are urgently needed.

Phage therapy

Could (bacterio)phages, the viruses of bacteria, help fight antibiotic-resistant bacteria [16–18]? A virus is a natural biological entity, consisting in essence of a molecular assemblage of nucleic acids (the genome) surrounded by proteins, that behaves as a genetic replicative parasite. Lytic phages attach to receptors on the surface of bacteria, inject their genetic material through the bacterial membrane and take over the bacterium's transcription and translation machinery to synthesize new phages. Finally, the bacterial cell wall is destroyed (lysed), releasing the newly assembled virions to the environment, where they can invade new bacteria. Importantly, phages are able to infect bacteria regardless of their susceptibility to antibiotics. Wherever bacteria are present, there are bound to be phages, generally in an order of magnitude higher than bacteria. With an estimated unit number of 10^{31}, phages are the most abundant biological lifelike constituents of our biosphere [19–21]. In fact, one could say that we live in an ocean of phages. But this does not automatically mean that all phages are safe at therapeutic concentrations. No phage-related nucleic acid sequence can be found in our genome, unlike the huge amount of human endogenous retroviral sequences, which make up 8–10% of the human genome [22,23]. Some phage-related polymerase gene sequences were identified in human mitochondrial DNA. It is common knowledge that mitochondria originated from *Rickettsia*-like ancestor bacteria that started a symbiotic relationship with prototype eukaryotic cells [24]. Phage DNA was likely introduced in the bacterial phase of the mitochondrion, at the time when the evolutionary split occurred between the prokaryotes and eukaryotes (endosymbiotic era), and does not constitute evidence for recent DNA exchange. Moreover, recent work suggests that even the eukaryotic nucleus itself is a viral import [25]. It is possible that phage sequences did enter the human genome, but were lost over time. In addition, entry into our germline may be irrelevant to the potential for causing harm, and we do not know how often phage DNA integrated into human somatic cells. One must also consider that the potential adverse effects of phages might not be caused by them acting as viruses. Researchers from the Hirszfeld Institute of Immunology and Experimental Therapy in Poland found phages to be constantly present in human and animal bodies [26], where they were shown to modulate immune functions [27] and interact with cancer cells [28]. It is virtually impossible for a phage to enter directly into a eukaryotic cell system and subsequently multiply since it requires prokaryotic-specific cell wall receptors and biochemical machinery for its attachment and replication (e.g., prokaryotic polymerases and tRNAs). However, we should also consider indirect ways for phages

to enter eukaryotes, no matter how far-fetched they may be. For example, theoretically, a phage could integrate into a plasmid, which could then transfer from a bacterium to a eukaryote.

According to most supporters, however, phage therapy has been proven safe through the massive application of lytic bacteriophages in humans in the past. We conclude that, although there are indications that phages are not harmful for eukaryotic organisms, more research is needed.

Today, a few laboratories and small and medium enterprises are developing phage cocktails or phage-based products for the treatment of bacterial infection [29]. This antibacterial therapeutic approach was first proposed by Felix d'Herelle almost a century ago. The first therapeutic application of phages probably occurred as early as 1919 in Paris, where d'Herelle used phages to treat patients suffering from bacterial dysentery [30]. Later, he founded the Laboratoire du Bactériophage in Paris, which produced five phage preparations for commercial use. They were marketed by the French company Robert et Carrière, which later was acquired by L'Oréal [31]. In the USA in the 1930s, pharmaceutical giants like Eli Lilly, Squibb & Sons (today Bristol-Myers Squibb) and the Swan–Myers division of Abbott Laboratories started marketing several phage preparations. Scientific uncertainties and the discovery and widespread marketing of antibiotics, however, relegated phage therapy to the history books in the western world. As such, the current 'knowledge' of the therapeutic effect of phages is mainly based on theoretical grounds, basic laboratory observations, animal models [32–37], safety studies in healthy humans [38,39] and decades of empirical medical experience [31,40–43]. These empirical data were mainly accumulated in the former Soviet Union and its eastern European satellite states, with an important role for the Eliava Institute of Bacteriophage, Microbiology and Virology in Tbilisi (Georgia), several institutes in Russia and the Hirszfeld Institute in Wroclaw (Poland). Phage therapy remained a valid therapeutic component in France until the early 1990s [44]. Unfortunately, the historical clinical data are not taken into account by regulators because it has not been validated according to current western regulatory standards. The emergence of MDR bacteria has caused a renewed interest in phage therapy in western Europe and the USA, as illustrated by an exponential increase in phage therapy-related papers in the medical literature (Figure 1).

Phages: not your regular medicinal products

Phages can be seen as bacteria's natural infectious agents. Up to 50% of bacterial mortality is thought to be due to phage-induced lysis [45]; hence, phages impose strong selection for bacteria resistance. However, lytic phages can only propagate by infecting and lysing bacteria, hence there is strong selection to overcome this resistance. This interaction leads to antagonistic coevolution, consisting of the repeated emergence of new phage infectivity and bacterial defense mutations [46–50]. Typically, coevolution results in continual increases in bacteria resistance and phage infectivity ranges, although recent work, including a study following real-time coevolution in soil [51], suggests that high costs associated with resistance may instead result in different, rather than greater, resistance mechanisms being selected through time [48,52]. In principle, coevolution between bacteria and phages could therefore allow the continual production of highly infectious phages that can overcome common bacterial defense mechanisms. However, it is important to emphasize that not all phages are lytic. Many integrate into bacterial genomes, and are propagated via bacterial reproduction [53]. Such lysogenic phages will themselves coevolve with each other [54], with bacteria and with other lytic phages, and the consequences of this for phage therapy are currently unclear. A recent study showed that *in vitro* coinfection of *Pseudomonas fluorescens* with multiple phages had no net effect of accelerating or slowing down

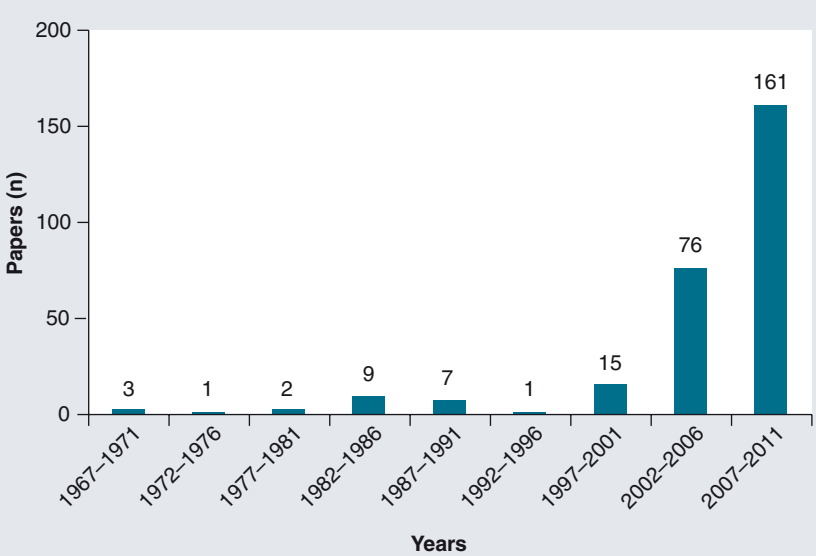


Figure 1. PubMed search results for 'phage therapy' or 'bacteriophage therapy' across time periods.

adaptation to the host through between-parasite conflict in the system [55]. It is thus tempting to speculate that phages act as 'evolving antibiotics' during real-time coevolution between therapeutic phages and infecting bacteria within patients. However, while real-time coevolution between bacteria and phages results in continual suppression of bacterial densities to some extent [51,56], the clinical significance of these relatively modest density directions is still unclear [57]. Phages do, however, play a major role in controlling bacterial densities in natural populations, and it is reasonable to assume that coevolution plays a role in this. For example, phages appear to be key players in ending cholera epidemics. Faruque *et al.* observed that seasonal epidemics of cholera inversely correlated with the prevalence of environmental cholera phages [58]. The removal of phages by conditions such as severe flooding might contribute to rendering water more conducive to human-to-human transfer of *Vibrio cholerae*. Phage amplification in cholera patients during a cholera epidemic likely contributed to increased environmental phage abundance, decreased load of environmental *V. cholerae* and, hence, the collapse of the epidemic. *In vivo* phage amplification in patients and subsequent phage infection in the environment could thus explain the self-limiting nature of seasonal cholera epidemics in Bangladesh [59].

It is clear that therapeutic phages are very different from classical (chemical, molecular) medicinal products such as antibiotics. Instead, they are natural biological entities that play an important role in maintaining equilibrium in bacterial populations of ecological environments, including humans. Hence, we should not see them as conventional stable medicinal products, but more as interactive and evolving antibacterial products, which could also be used in combination (synergy) with antibiotics [60]. The coevolutive aspect of the phage–bacterium couplet, which is essential for sustainable phage therapy, is often neglected.

However, there are potential negative consequences of this coevolutionary potential. For example, coevolution has been shown to drive the evolution of bacterial mutation rates in laboratory populations of the bacterium *P. fluorescens*. A quarter of the bacterial populations coevolving with phages had rapidly (i.e., in less than 200 generations) acquired mutations that resulted in ten- to 100-fold increases in mutation rates, whereas no significant change in mutation rates was observed in the absence of phages [61]. Given the increase in evolvability of mutator bacteria (e.g., elevated rates of resistance evolution to antibiotics), evolvable phages may have unknown net consequences on disease severity. Phage therapy should not be implemented widely and without limitation, without first determining these consequences through real-time experimental evolution studies. In the end, natural phages could prove useful, but maybe only in specific (niche) clinical contexts and under certain conditions (e.g., dosage).

Phage therapy fits well in the emerging field of Darwinian medicine (in contrast to a classical mechanistic – man as a machine – view) [62,63], whereby the insights into evolution are fully taken into account, but it is less compatible with our actual western drug development and marketing model.

Hurdles in the current medicinal product development & marketing model

This section discusses the problems encountered when trying to reintroduce traditional phage therapy in modern medicine.

An analysis of the current European regulatory framework [64] and multiple discussions with experts and the relevant competent authorities revealed that, although the development and marketing of phage medicinal products (including good manufacturing practice production, preclinical and Phase I, II and III clinical trials and centralized marketing authorization) is technically possible, in practice it is not compatible with traditional (sustainable) phage therapy [65].

The cost of conventional medicinal product development & marketing (millions of Euros) necessitates strong intellectual property protection, but today, for natural phages, this protection is fragile

Recently, the ruling in a US court in a case between the Association of Molecular Pathology and the US Patent and Trademark Office invalidated seven patents claiming genes and genetic diagnostic methods held by Myriad Genetics [66]. Although related to genes, this decision opens the discussion about the ability to patent naturally occurring organisms such as phages.

In patent law, an invention is considered to be new if it is not part of the state of the art. This means that a phage or a phage cocktail claimed in a patent should never have been isolated or produced before. The literature with respect to phages as natural entities to treat human bacterial infections is enormous. In addition, clinical studies using phages performed in

the eastern part of Europe have recently been translated into English (e.g., [40]). Therefore, many natural phages and their uses have been disclosed over the past century. European law allows the patenting of known substances, such as natural phages, for use in a medical method, provided that such use is new, meaning that such use may not be comprised in the state of the art. In the USA, several patents for phages used in the food sector were granted, such as US7507571 (food additive), claiming "an isolated bacteriophage of a bacteriophage strain selected from a [specific] group, [somewhere] deposited under a [specific] accession number, together with variants thereof, wherein said variants retain the phenotypic characteristics of said deposited bacteriophages and wherein said bacteriophages, and variants thereof, have lytic activity against *Listeria monocytogenes* strains" [67]. More important for therapeutic use is the US patent 7459272 of Intralytix, Inc., claiming "a method for reducing the risk of bacterial infection or sepsis in a person colonized with pathogenic bacteria comprising treating the colonized person with a pharmaceutical composition containing bacteriophage of one or more strains which produce lytic infections in said pathogenic bacteria." In 2001, a European patent application (EP1250143 A2) was filed, claiming "a method for reducing the risk of bacterial infection or sepsis in a susceptible patient by treating the susceptible patient with a pharmaceutical composition containing bacteriophages of one or more strains which produce lytic infections in pathogenic bacteria," but this application was withdrawn in 2004. Only recently, "a method for production of compositions of bacteriophages" was claimed in the USA by Phage Biopharm, LLC (US7588929). No European counterpart has been published yet. Another interesting patent is the US patent 7758856 (Biocontrol, Ltd) claiming "a composition for treating a bacterial biofilm," as well as "a method for treating a biofilm infection." A similar patent owned by the UK Health Protection Agency has been granted in Europe (EP1587520 B1).

Diverging views between Europe and the USA exist on the patenting of biological material. Next to the requirements of novelty, inventive steps and industrial applicability (which are the same for Europe and the USA), in order to be patentable in Europe, a certain technical intervention is needed to isolate the phage from its natural environment, and the isolated phage needs to be properly characterized. However, this 'technical intervention' has basically been known since the 1920s, and the requirement that the phage 'needs to be well characterized' seems obvious and is technically not particularly hard to meet [68]. In the USA, phages claimed in a patent need to have markedly different characteristics from their counterparts found in nature. But, for natural exclusively lytic phages – our object of concern here – they simply are the ones found in nature. It seems as if only genetically modified phages can agree with the US statement. While 'manipulated' or engineered phages certainly have potential applications (which are patentable), given the growing public concern and awareness over the potential health and environmental risks of genetically modified organisms, they are unlikely to obtain licensing approval in the near future.

Phage-encoded proteins such as cell wall-degrading endolysins [69] will be marketed a few years from now in the food industry, the veterinary field and possibly in medicine. They will select resistance, but presumably and hopefully at a slower pace than antibiotics. Of course, these phage-derived products are not capable of self-replicating and evolving in the infectious site.

In this paper, we focus on natural phages simply because of their natural intrinsic bacterial coevolutionary aspect making them suitable for flexible therapeutic applications. Patents claiming natural phages are fragile, and 'inventing around' (making an invention that accomplishes the same thing as the original patented invention but does not infringe the patented invention) also seems to be very difficult [70].

These intellectual property (IP) issues do not stimulate investment (of venture capital), for the actual paradigm is 'no IP protection, no investment'. However, the renewed interest in natural phages as therapeutic agents might trigger scientists' and entrepreneurs' creativity in defining the contours of appropriate patent claims for phages or, even better, because there are good reasons for not patenting certain natural substances, considering a new kind of IP instrument. New ideas on IP protection should not be based on the existing classical model, but on a broader 'new' philosophy in relation to sustainable economic and industrial development, as advocated by Petrella and Sachs [71,72]. Petrella states that, today, "being competitive" is no longer a tool for increased development, but an aim in itself [71]. This increasingly implies that the possession of patents, often as strategic weapons, is more important (in the short term)

than owning a truly functional innovative technology. This kind of attitude tends to block the development of new approaches such as phage therapy. The patent tragedy is indeed exemplified by the millions of AIDS victims who died while drug treatments existed and raises deep questions about global IP rights. How can the benefits of a global patent system that provides incentives for innovation and continuous development be combined with an assurance that the targeted people (rich and poor) gain access to the medical care they need and have rights to [73]?

Therefore, the Group of Lisbon, led by Petrella, proposed an evolution to world cooperative governance, which is based on a global contract that requires that each decision should be linked to the fact that each person should have access to basic livelihoods [71], including health access, which is actually often blocked by our outdated economic model. As such, phage therapy could be developed under the umbrella of, for example, the WHO. The WHO recognizes the importance of the worldwide antibiotic resistance issues [101] and is discussing new incentives to push the pharmaceutical industry to launch new research and development projects. Could phage therapy be one of them?

The time frames for conventional medicinal product development & marketing (years) are not compatible with a flexible, tailor-made & sustainable phage therapy concept

Phage therapy depends upon safe and well-defined phages, but is it really necessary to produce and market them in the same way as conventional medicinal products?

In 2009, a phage cocktail, BFC-1, which targeted the most prevalent MDR *P. aeruginosa* and MRSA bacteria in the burn wound center of the Queen Astrid Military Hospital in Brussels (Belgium), was produced. The cocktail consisted of two phages against *P. aeruginosa* and one against *S. aureus*. It was produced on a small scale and in concordance with certain relevant quality and safety standards (e.g., sterility, apyrogenicity, pH, adequate shelf life and stability). In addition, the phages were shown to be exclusively lytic and were characterized at the genomic and proteomic level. This specific production process was published in 2009 by Merabishvili *et al.* [68]. As the authors did not consider phages to be conventional medicinal products, the phage cocktail was not produced in concordance with the requirements of the EU medicinal product regulation. After approval by a leading Medical Ethical Committee (of the Free University of Brussels), phage cocktail BFC-1 was applied in a small pilot study in the burn unit of the Queen Astrid Military Hospital in Brussels. This small trial was discussed in a recent review by Kutter and colleagues [43]. No adverse events or side effects were observed.

However, the European Commission stated recently that EU's legislation on medicinal products does not define specific requirements related to bacteriophage therapy or medicines composed of bacteriophages because it considers that the existing regulatory framework is adequate for bacteriophage therapy. There is thus no need for a specific set of documentation for bacteriophage therapy [74]. We do not share this opinion for the reasons discussed below.

To exploit the main advantage of phages over classical 'static' drugs such as antibiotics, and more specifically their capacity to rapidly (in a matter of days to weeks) evolve to target emerging (phage-resistant) pathogenic bacterial strains, phage cocktails should not be submitted to the conventional long medicinal product development and licensing pathway. Even if the EMA would eventually adapt its rules in a similar manner to what they did for updated seasonal influenza vaccines, which are annually licensed [75], development times of many months are still much too long in view of the enormous challenges related to rapidly progressing bacterial resistance. The real power of phage therapy lies in the fact that the search for a potent natural phage and the preparation of a classic galenic preparation (e.g., physiological water or a basic ointment) containing phages is practically feasible in the time frame of days to weeks. In traditional phage therapy, new therapeutic phages are usually selected from environmental sources such as raw sewage water or isolated from clinical specimens from infected patients (Figure 2). Georgian and Polish phage therapy centers are keeping extensive therapeutic phage collections, which are regularly enriched with new phages, thus widening the host range of the collection. Ineffective phages can be 'trained', a term indicating the *in vitro* selection of phage mutants that exhibit an increased infectivity range. As such, it is possible to obtain potent lytic phages against problematic enteroaggregative *E. coli* strains [76] in a matter of days, for example. Theoretically, they could thus have been used to

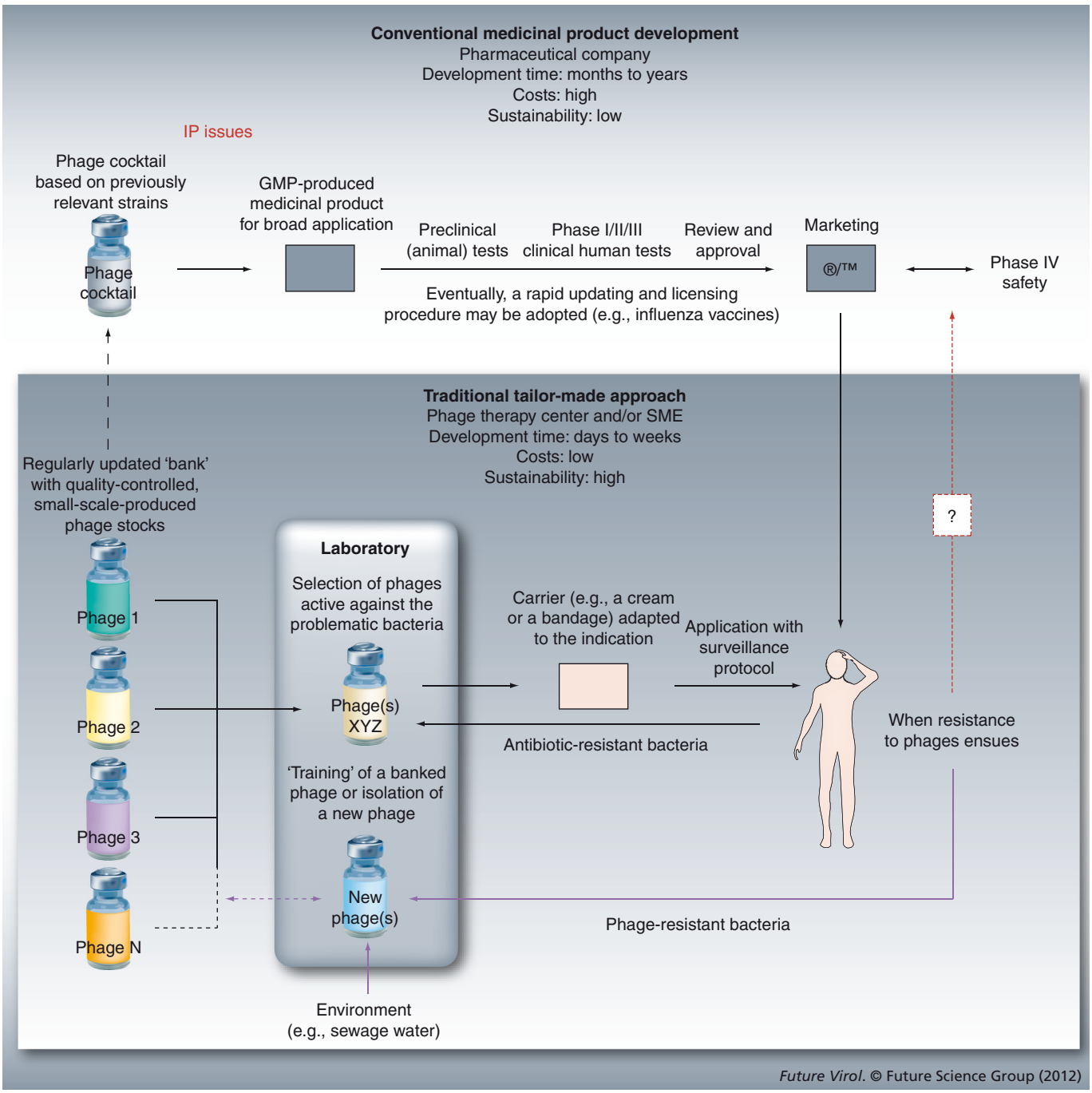


Figure 2. Two phage therapy concepts. IP issues may hamper pharmaceutical companies in the worldwide marketing of generic phage preparations. The long and expensive regulatory pathways form insurmountable obstacles for eventual nonprofit phage therapy centers or SMEs, which opt for a tailor-made concept, and for institutions that would like to use inexpensive phages for commercially unattractive applications (e.g., in developing countries) [65].
GMP: Good manufacturing practice; IP: Intellectual property; SME: Small and medium enterprise.

help control the O104:H4 outbreak that caused the death of 50 patients in Germany [9,10]. In this context, an O104:H4 phage preparation that takes months to years to develop, produce and register is ineffective. As phages are species- and often even strain-specific, it is very likely that current O104:H4-specific phage preparations will not be active against future epidemic enteroaggregative *E. coli* strains. Provided that future problematic bacteria are broadly known, some 'broad-spectrum' cocktails could be developed in advance and used as the first-line answer to acute healthcare problems (e.g., bioweapons). Some cocktails will inevitably fail due to the greater biodiversity outside of the laboratory, and the ones that initially work will need to

be regularly updated due to the emergence of resistance. In a recent study, it was shown that *P. aeruginosa* challenged *in vitro* with a cocktail of four potent phages swiftly developed resistance to all four phages [Hall AR, De Vos D, Friman VP, Pirnay JP, Buckling A. Effects of sequential and simultaneous application of bacteriophages on populations of *Pseudomonas aeruginosa in vitro* and in waxmoth larvae (2012), Submitted]. We are currently discussing our viewpoint with EMA's Innovation Task Force (ITF). The ITF has the competence to facilitate the informal exchange of information and the provision of guidance early in the development process of medicinal products. Our objectives are to develop a specific framework (e.g., realistic production and documentation requirements) that allows a timely (rapid) supply of tailor-made productions of natural bacteriophages to patients.

Responsible & sustainable phage therapy is not compatible with current pharmacoeconomic models

Acceptable IP protection and development and licensing procedures were available for antibiotics. They did not prevent the overuses and misuses that gave rise to the current antibiotic resistance crisis. Solving the aforementioned IP and development issues will thus not necessarily lead to rational and sustainable phage therapy. The question is, how can responsible and limited use be promoted? It is very doubtful that this will be compatible with actual economic incentives. Even world cooperative governance will provide no guarantees, as the primary goal of organizations such as the WHO is to limit infections, not to support sustainable approaches.

It is our opinion that, ultimately, economic models will need to be radically reshaped in order to cater for more sustainable approaches such as phage therapy.

Current state

The tailor-made approach and sustainable nature of traditional phage therapy and IP issues may hamper pharmaceutical companies in the worldwide marketing of generic phage preparations. Nonprofit/public institutions such as (university) hospitals that would like to develop flexible and sustainable tailor-made (i.e., to an outbreak) phage therapy and are not necessarily disheartened by the IP issues and the subsequent uncertainty of large profits are generally unable to generate the necessary funding. In addition, the prescribed medicinal product development and licensing pathways cancel the advantages of phage therapy over antibiotics. It is thus difficult to reconcile a flexible and sustainable phage therapy concept with the current (western) medical and pharmaceutical environment (**Figure 2**). As a result of this conundrum, only local and sporadic phage applications have been performed in the western world to date, often based on individual approval governed within the 'Declaration of Helsinki' framework [102]. In Poland, an EU member state, a specific national adaptive regulation, based on the Declaration of Helsinki, was issued to regulate phage therapy. A medical doctor is allowed to apply phage therapy where proven therapeutic methods do not exist or have been ineffective (e.g., in MDR infections) and provided that the patient or their legal representative gives informed consent. In France, Alain Dublanchet, a veteran of phage therapy, occasionally applies phages in hopeless osteomyelitis cases [65]. In Australia, phage therapy was recently applied under the umbrella of 'compassionate use' for the successful treatment of refractory *P. aeruginosa* urinary tract infection in a cancer patient [77].

Conclusion

Phages are not straightforward inanimate and stable substances, but evolvable and natural biological entities. Future sustainable phage therapy concepts should fully acknowledge the potential of the coevolutionary aspect of the phage–bacterium couplet. Only then can the inherent potential of phages as natural biological bacterium controllers be put to use. Indeed, bacteria will inevitably become resistant to phages, but due to the continuously ongoing arms race/competition between the two protagonists, specific phages that are able to infect the formerly resistant bacterial strains can be expected to quickly emerge. However, more experimental evolution studies are necessary to determine the potential negative evolutionary consequences of unlimited phage therapy.

The existing pharmaceutical regulatory framework and business models are not compatible with a dynamic and sustainable phage therapy concept. The actual economic models reduce pharmaceutical companies to 'common button' producers neglecting their main societal role: providing people with adequate products for better health. Therefore, a suitable environment for phage therapy should be developed. Fundamental changes of mentality in the medical and pharmaceutical environment (e.g.,

towards patentability and restrictive licensing) are essential for a successful introduction of phage therapy in modern (future) medicine. We need to radically reshape our (pharmaceutical) economic models to cater for more sustainable approaches that are beneficial for human survival.

Phage therapy fits well in the new emerging field of Darwinian medicine, where the insights of evolution are fully taken into account. Viruses, among which are phages, were involved in the origin of life itself and play a major role in biological evolution [78–82]. Hopefully, they will play a role in the future control of bacterial disease. We consider our plea for a more realistic approach to phage therapy, which takes into account the coevolutionary aspect of the bacterium and its phage, to be scientifically sound. We must learn from the errors that contributed to the rise of antibiotic resistance. We hope to foster this vision in collaboration with the competent authorities and responsible economic actors, as only a common effort will make it a (direly needed) reality.

Future perspective

In the short term, we predict the setting up of credible studies to gather the required data with regard to the efficacy and evolutionary consequences of phage therapy. These studies could be chaperoned by health protection agencies such as the European CDC.

In the medium term, we predict the development of a specific framework, in collaboration with the EMA's ITF (or with the US FDA), with realistic production and documentation requirements that allow a timely supply of safe, tailor-made natural bacteriophages.

In the long term, we predict the radical reshaping of our (pharmaceutical) economic models to cater for more sustainable approaches. Phage therapy could be developed under the umbrella of the WHO.

Executive summary

Spreading antibiotic resistance: a universal threat

- Overuse and misuse of antibiotics caused the emergence of organisms that are resistant to these medicinal products, leading to increased morbidity and mortality and increased healthcare costs.
- Because new antibiotics have become of limited use and are thus less profitable, pharmaceutical companies are reluctant to invest in the research and development of new antibiotics.

Phage therapy

- Phage therapy – the use of the viruses of bacteria to fight bacterial infection – was first advocated by Felix d'Herelle in 1919.
- Due to the advent of antibiotics and scientific controversies, phage therapy was abandoned in the western world.
- The current antibiotic resistance crisis has caused a renewed interest in phage therapy.

Phages: not your regular medicinal products

- Phages are very different from classical (chemical molecular) medicinal products.
- Phages are natural biological entities that coevolve with and control bacteria in the environment, including humans, which is the basis of sustainable phage therapy.
- There might also be potential negative consequences of bacterial phage coevolution.

Hurdles in the current medicinal product development & marketing model

- When trying to introduce traditional sustainable phage therapy in modern medicine, one is confronted with three issues:
  - The cost of conventional medicinal product development and marketing (millions of Euros) necessitates strong intellectual property protection, but today, for natural phages, this protection is fragile;
  - The time-frames for conventional medicinal product development and marketing (years) are not compatible with a flexible, tailor-made and sustainable phage therapy concept;
  - Responsible and sustainable phage therapy is not compatible with current pharmacoeconomic models.

Current status

- Only local and sporadic phage applications are performed in the western world, often based on individual approval governed within the 'Declaration of Helsinki' framework.

Conclusion

- Future sustainable phage therapy concepts should fully acknowledge the potential of the coevolutionary aspect of the phage–bacterium couplet.
- More research is needed to determine the potential negative coevolutionary consequences of unlimited phage therapy.
- Our (pharmaceutical) economic models need to be radically reshaped to cater for more sustainable approaches that are beneficial for human survival.

Financial & competing interests disclosure

The authors thank the FWO Vlaanderen ('PhageBiotics' research community grant WO.022.09) for its support. J-P Pirnay and M Merabishvili were supported by grant MED 12 of the Royal High Institute for Defence. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 10(12), S122–S129 (2004).
2. Bush K, Courvalin P, Dantas G *et al.* Tackling antibiotic resistance. *Nat. Rev. Microbiol.* 9(12), 894–896 (2011).

▪▪ **A group of 30 scientists from academia and industry gathered to explore how the problem of antibiotic resistance might best be addressed.**

3. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J. Infect. Dis.* 197(8), 1079–1081 (2008).
4. Jones A, Morgan D, Walsh A *et al.* Importation of multidrug-resistant *Acinetobacter* spp infections with casualties from Iraq. *Lancet Infect. Dis.* 6(6), 317–318 (2006).
5. Kumarasamy K, Toleman MA, Walsh TR *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 10(9), 597–602 (2010).
6. Pirnay JP, Bilocq F, Pot B *et al. Pseudomonas aeruginosa* population structure revisited. *PLoS ONE* 4(11), E7740 (2009).
7. Michel M, Gutmann L. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: therapeutic realities and possibilities. *Lancet* 349(9069), 1901–1906 (1997).
8. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* 375(9725), 1557–1568 (2010).
9. Struelens MJ, Palm D, Takkinen J. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O104:H4 outbreak: new microbiological findings boost coordinated investigations by European public health laboratories. *Euro Surveill.* 16(24), 1–3 (2011).
10. Rasko DA, Webster DR, Sahl JW *et al.* Origins of the *E. coli* strain causing an outbreak of haemolytic–uremic syndrome in Germany. *N. Engl. J. Med.* 365(8), 709–717 (2011).
11. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. *Clin. Microbiol. Rev.* 15(4), 647–679 (2002).
12. Baquero F, Coque TM, Canton R. Antibiotics, complexity and evolution. *ASM News* 69, 547–552 (2003).
13. Kümmerer K, Henninger A. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin. Microbiol. Infect.* 9(12), 1203–1214 (2003).
14. Kümmerer K. Resistance in the environment. *J. Antimicrob. Chemother.* 54(2), 311–320 (2004).
15. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. *Nat. Rev. Microbiol.* 8(4), 251–259 (2010).
16. Maura D, Debarbieux L. Bacteriophages as twenty-first century antibacterial tools for food and medicine. *Appl. Microbiol. Biotechnol.* 90(3), 851–859 (2011).
17. Thiel K. Old dogma, new tricks – 21st century phage therapy. *Nat. Biotechnol.* 22(1), 31–36 (2004).

▪▪ **Provides an overview of how biotechnology is trying to develop phage therapy.**

18. Kutateladze M, Adamia R. Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends Biotechnol.* 28(12), 591–595 (2010).
19. Bergh O, Borsheim KY, Bratbak G, Heldal M. High abundance of viruses found in aquatic environments. *Nature* 340(6233), 476–468 (1989).
20. Fuhrman JA. Marine viruses and their biogeochemical and ecological effects. *Nature* 399(6736), 541–548 (1999).
21. Hendrix RW. Bacteriophages: evolution of the majority. *Theor. Popul. Biol.* 61(4), 471–480 (2002).
22. Lander ES, Linton LM, Birren B *et al.* Initial sequencing and analysis of the human genome. *Nature* 409(6822), 860–921 (2001).
23. Mi S, Lee X, Li X *et al.* Syncitin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403(6771), 785–789 (2000).
24. Spelbrink JN, Li FY, Tiranti V *et al.* Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat. Genet.* 28(3), 223–231 (2001).
25. Bell PJL. Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus? *J. Mol. Evol.* 53(3), 251–256 (2001).
26. Górski A, Wazna E, Dabrowska BW, Dabrowska K, Switała-Jeleń K, Miedzybrodzki R. Bacteriophage translocation. *FEMS Immunol. Med. Microbiol.* 46(3), 313–319 (2006).
27. Gorski A, Dabrowska K, Switala-Jeleń K *et al.* New insights into the possible role of bacteriophages in host defense and disease. *Med. Immunol.* 2(1), 2 (2003).
28. Budynek P, Dabrowska K, Skaradziński G, Górski A. Bacteriophages and cancer. *Arch. Microbiol.* 192(5), 315–320 (2010).
29. Monk AB, Rees CD, Barrow P, Hagens S, Harper DR. Bacteriophage applications: where are we now? *Lett. Appl. Microbiol.* 51(4), 363–369 (2010).
30. d'Herelle F. Sur le rôle du microbe bactériophage dans la typhose aviaire. *C. R. Acad. Sci.* 169, 932–934 (1919).
31. Sulakhvelidze A, Alavidze Z, Morris JG. Bacteriophage therapy. *Antimicrob. Agents Chemother.* 45(3), 649–659 (2001).
32. Biswas B, Adhya S, Washart P *et al.* Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect. Immunol.* 70(3), 204–210 (2002).
33. Chibani-Chenouffi S, Sidoti J, Brüttin A, Kutter E, Sarket S, Brüssow H. *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. *Antimicrob. Agents Chemother.* 48(7), 2558–2569 (2004).
34. Wills QF, Kerrigan C, Soothill JS. Experimental bacteriophage protection against *Staphylococus aureus* abscess in a rabbit model. *Antimicrob. Agents Chemother.* 49(3), 1220–1221 (2005).
35. Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. *Burns* 32(5), 644–646 (2006).
36. McVay CS, Velasquez M, Fralick JA. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model.

Antimicrob. Agents Chemother. 51(6), 1934–1938 (2007).

37. Debarbieux L, Leduc D, Maura D *et al.* Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. *J. Infect. Dis.* 201(7), 1096–1104 (2010).
38. Brüttin A, Brüssow H. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.* 49(7), 2874–2878 (2005).
39. Rhoads DD, Wolcott RD, Kuskowski MA, Wolcott BM, Ward LS, Sulakvelidze A. Bacteriophage therapy of venous leg ulcers in humans: results of a Phase I safety trial. *J. Wound Care* 18(6), 237–243 (2009).
40. Chanishvili N. *A Literature Review of the Practical Application of Bacteriophage Research*. Sharp R (Ed.). Eliava Institute, Georgia (2009).

▪ **Reports, in English, the results of screening for bacteriophage-related data in over 5000 scientific documents (papers, books and PhD theses) available from the library of the Eliava Institute in Tbilisi (Georgia).**

41. Dabrowska K, Switala-Jelen K, Opolski A, Weber-Dabrowska B, Gorski A. Bacteriophage penetration in vertebrates. *J. Appl. Microbiol.* 98(1), 7–13 (2003).
42. Gorski P, Miedzybrodski R, Borysowski J *et al.* Bacteriophage therapy for the treatment of infections. *Curr. Opin. Investig. Drugs* 10(8), 766–774 (2009).
43. Kutter E, De Vos D, Gvasalia G *et al.* Phage therapy in clinical practice: treatment of human infections. *Curr. Pharm. Biotechnol.* 11(1), 69–86 (2010).
44. Dublanchet A. *Des Virus Pour Combattre les Infections: La Phagothérapie: Renouveau d'un Traitement au Secours des Antibiotiques.* Favre SA (Ed.). Lausanne, Switzerland (2009).
45. Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* 64(1), 69–114 (2000).
46. Buckling A, Rainey PB. Antagonistic coevolution between a bacterium and a bacteriophage. *Proc. Biol. Sci.* 269(1494), 931–936 (2002).
47. Bohannan BJM, Lenski RE. Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.* 3, 362–377 (2000).
48. Hall A, Scanlan PD, Morgan AD, Buckling A. Host–parasite coevolutionary arms races give way to flcutuating selection. *Ecol. Lett.* 14, 635–642 (2011).
49. Mizoguchi K, Morita M, Fischer CR, Yoichi M, Tanji Y, Unno H. Coevolution of bacteriophage PP01 and *Escherichia coli* O157:H7 in continuous culture. *Appl. Environ. Microbiol.* 69(1), 170–176 (2003).
50. Stern A, Sorek R. The phage–host arms race: shaping the evolution of microbes. *Bioessays* 33(1), 43–51 (2011).
51. Gómez P, Buckling A. Bacteria–phage antagonistic coevolution in soil. *Science* 332(6025), 106–109 (2011).
52. Agrawal A, Lively CM. Infection genetics: gene-for-gene versus matching-alleles models and all points in between. *Evol. Ecol. Res.* 4(1), 79–90 (2002).
53. Calendar RL. *The Bacteriophages*. Oxford University Press, UK (2005).
54. Refardt D. Within-host competition determines reproductive success of temperate bacteriophages. *ISME J.* 5(9), 1451–1460 (2011).
55. Hall AR, Scanlan PD, Leggett HC, Buckling A. Multiplicity of infection does not accelerate infectivity evolution of viral parasites in laboratory microcosms. *J. Evol. Biol.* 25(2), 409–415 (2012).
56. Buckling A, Hodgson DJ. Short-term rates of parasite evolution predict the evolution of host diversity. *J. Evol. Biol.* 20(5), 1682–1688 (2009).
57. Levin BR, Bull JJ. Population and evolutionary dynamics of phage therapy. *Nat. Rev. Microbiol.* 2(2), 166–173 (2004).

▪ **Reviews the population and evolutionary dynamics of bacterial–phage interactions that are relevant to phage therapy and prophylaxis.**

58. Faruque SM, Naser IB, Islam MJ *et al.* Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proc. Natl Acad. Sci. USA* 102(5), 1702–1707 (2005).

▪▪ **Good example of the major role that phages play in controlling bacterial densities in natural populations.**

59. Faruque SM, Islam MJ, Ahmad QS *et al.* Self-limiting nature of seasonal cholera epidemics: role of host-mediated amplification of phage. *Proc. Natl Acad. Sci. USA* 102(17), 6119–6124 (2005).
60. Comeau AM, Tétart F, Trojet SN, Prère MF, Krish HM. Phage–antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS ONE* 2(8), E799 (2007).
61. Pal C, Maciá MD, Oliver A, Schachar I, Buckling A. Coevolution with viruses drives the evolution of bacterial mutation rates. *Nature* 450(7172), 1079–1081 (2007).

▪ **Provides evidence that, *in vitro*, phages can increase the evolvability of mutator bacteria, leading to unknown net consequences on disease severity.**

62. Williams GC, Nesse RM. The dawn of Darwinian medicine. *Q. Rev. Biol.* 66(1), 1–22 (1991).
63. Nesse RM. On the difficulty of defining disease: a Darwinian perspective. *Med. Health Care Philos.* 4(1), 37–46 (2000).
64. Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP. European regulatory conundrum of phage therapy. *Future Microbiol.* 2(5), 485–491 (2007).
65. Pirnay JP, De Vos D, Verbeken G *et al.* The phage therapy paradigm: prêt-à-porter or sur-mesure? *Pharm. Res.* 28(4), 934–937 (2010).
66. Akst J. Key cancer patents killed. *The Scientist*, 30 March (2010).
67. FDA. FDA 21 CFR Part 172: food additives permitted for direct addition to food for human consumption; bacteriophage preparation. *Fed. Reg.* 71(160), 47729–47732 (2006).
68. Merabishvili M, Pirnay JP, Verbeken G *et al.* Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. *PLoS ONE* 4(3), E4944 (2009).
69. Callewaert L, Walmagh M, Michiels CW, Lavigne R. Food applications of bacterial cell wall hydrolases. *Curr. Opin. Biotechnol.* 22(2), 164–171 (2011).
70. Van Overwalle G. Intellectual property. Turning patent swords into shares. *Science* 230(6011), 1630–1631 (2010).
71. The Group of Lisbon. *Limits to Competition*. MIT Press, MA, USA (1995).
72. Sachs JD. *Common Wealth: Economics for a Crowded Planet.* Penguin, UK (2008).

▪ **Provides an interesting roadmap to sustainable and equitable global prosperity.**

73. So AD, Gupta N, Brahmachari SK *et al.* Towards new business models for R&D for novel antibiotics. *Drug Resist. Updat.* 14(2), 88–94 (2011).
74. Verbeken G, Pirnay J-P, De Vos D *et al.* Optimizing the European regulatory frame for sustainable bacteriophage therapy in human medicine. *Arch. Immunol. Ther. Exp.* (2012) (In Press).
75. Wood JM, Levandowski RA. The influenza vaccine licensing process. *Vaccine* 21(16), 1786–1788 (2003).
76. Maura D, Morello E, du Merle L, Bomme P, Le Bouguénec C, Debarbieux L. Intestinal

colonization by enteroaggregative *Escherichia coli* supports long-term bacteriophage replication in mice. *Environ. Microbiol.* doi:10.1111/j.1462-2920.2011.02644.x (2011) (Epub ahead of print).

77. Khawaldeh A, Morales S, Dillon B *et al.* Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. *J. Med. Microbiol.* 60(11), 1697–1700 (2011).
78. Villarreal LP. Overall issues of viruses and host evolution. In: *Viruses and the Evolution of Life*. Villareal LP (Ed.). ASM Press, WA, USA, 1–28 (2005).
79. Villarreal LP, Witzany G. Viruses are essential agents within the roots and stem of the tree of life. *J. Theor. Biol.* 262(4), 698–710 (2010).
80. Bamford DH. Do viruses form lineages across different domains of life? *Res. Microbiol.* 154(4), 231–236 (2003).
81. Forterre P. Genomics and early cellular evolution, the origin of the DNA world. *C. R. Acad. Sci. III* 324(12), 1067–1076 (2001).
82. Forterre P. The origin of viruses and their possible role in major evolutionary transitions. *Virus Res.* 117(1), 5–16 (2006).

Websites

101. WHO. Policy Package to Combat Antimicrobial Resistance. www.who.int/world-health-day/2011/presskit/WHDIntrototobriefs.pdf?bcsi_scan_3c79e7817cdc4fd7=0&bcsi_scan_filename=WHDIntrototobriefs.pdf (Accessed January 2012)
102. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. www.wma.net/en/30publications/10policies/b3/index.html (Accessed January 2012)

Copyright holders

3.1.3 Paving a regulatory pathway for bacteriophage therapy

I. Huys, J.P. Pirnay, R. Lavigne, S. Jennes, D. De Vos, M. Casteels, G. Verbeken

Embo reports. 2013; 14(11):951-954

International scientific journal

EMBO reports

science & society

Paving a regulatory pathway for phage therapy

Europe should muster the resources to financially, technically and legally support the introduction of phage therapy

Isabelle Huys, Jean-Paul Pirnay, Rob Lavigne, Serge Jennes, Daniel De Vos, Minne Casteels & Gilbert Verbeken

The increasing resistance of bacteria to antibiotics is a significant threat to human health and is a direct result of the excessive and improper use of these drugs. In 2007, multidrug-resistant bacterial strains infected more than 400,000 people in Europe and 25,000 patients died from the infections [1]. 'Superbugs' also have considerable economic impact: extra hospital costs and related productivity losses amount to more than €1.5 billion per year in the European Union. In the USA, infections caused by multidrug resistant bacteria lead to US$20 billion in additional health-care costs and US$35 billion societal costs annually [2]. The situation is about to get worse, as there are only a few drugs left to treat multidrug-resistant bacterial strains, and the first strains that are resistant to even these last-resort antibiotics have already emerged. Moreover, there is a dearth of genuinely novel antibiotics in the development pipeline.

"...there are only a few drugs left to treat multidrug-resistant bacterial strains, and the first strains that are resistant to even these last-resort antibiotics have already emerged"

Various proposals have been made to address the problem. These range from the more-prudent use of existing antibiotics or better hygiene, to providing incentives to the pharmaceutical industry to develop novel drugs. In addition, the use of bacteriophages, or phage therapies, to kill specific pathogens without harming the majority of harmless, commensal bacteria has received increasing attention during the past decade, but little has been done to capitalize on this interest and implement phage therapies in the clinic.

The application of bacteriophages to treat infection dates back to around the 1920s. Today, phage therapies are routinely used in countries such as Georgia and Poland, but countries in western Europe abandoned such therapy after the introduction of antibiotics. Only a handful of clinical trials are ongoing and some are taking place in countries where European regulatory standards do not apply. Elsewhere, phage therapies are only applied sporadically in specialized medical centres for the *ad hoc* treatment of patients with severe infections. At this time, the greatest hurdle to the medical use of bacteriophages in Europe is the lack of an appropriate regulatory framework that appreciates the concept and specifics of this approach to support its application in the clinic. Part of the problem is that whether phage therapies are medicinal products or something completely different is unclear under current European legislation. Implementation and regulation of their use is therefore challenging.

The current legal framework for the use of medicines in Europe is mainly dictated by European directive 2001/83/EG, which outlines the European Community code relating to medicinal products for human use. This directive was passed into law more than 10 years ago. It defines any substance or combination of substances used to treat or prevent disease in humans as human medicinal products and, therefore, makes them subject to specific requirements relating to safety, quality and efficacy. How phage therapies should be defined remains in question.

"...the greatest hurdle to the medical use of bacteriophages in Europe is the lack of an appropriate regulatory framework that appreciates [its] concept and specifics..."

Bacteriophages are viruses that specifically attack bacteria and can be used to control, treat or prevent infectious diseases (Sidebar A). By controlling bacterial overgrowth, bacteriophages can re-equilibrate the host–bacteria balance and consequently they can indirectly restore physiological functions and boost the immune system. According to the definitions in the directive and the national legislation based on it, bacteriophages could be considered to be human medicinal products. The consequences of classifying them in this way would be far-reaching: phage therapies would require assessment in large clinical studies to demonstrate safety and efficacy. A strength of phage therapies is that they can be tailored to each patient and to each patient's bacterial infection. This flexibility is not fully compatible with the approach of the directive. In fact, bacteriophages are not mentioned in the current legislation, and the technical assistance or documentation that could be used to prepare a regulatory dossier does not exist.

Nevertheless, if we are to introduce phage therapy into clinical practice, they must be regulated according to the directive. To address its limitations, therefore, and in order to draft (Sidebar A) appropriate regulatory protocols for use, it is first necessary to define under which category of human medicinal products bacteriophages fall. The first category includes conventional small molecules or synthetic human medicinal drugs, such as aspirin, that can be described and researched in a standardized manner. From a functional point of view, such products are not comparable to bacteriophages because they operate in entirely different ways. Bacteriophages kill their specific bacterial host cells through bacterial lysis, which causes the release of new bacteriophage virions. When the targeted bacterial density drops below the detection threshold, the bacteriophages are removed by the reticuloendothelial system and the therapeutic intervention becomes self-terminating. Also unlike standard drugs, bacteriophages mutate and co-evolve with their host bacteria, an evolutionary 'arms race'.

"Isolating a bacteriophage to combat the infection, preparing a therapeutic dose and administering it to the patient needs to be done within days"

The second category of biological human medicinal products, which includes vaccines, seems more suitable, but it does not encapsulate all the features of phage therapies. From a general structural point of view, a bacteriophage is a protein-encapsulated nucleic acid genome. Moreover, they might be collected from a biological source, for example released from bacteria or collected from a patient's tissues or fluids (for instance from wounds) or from wastewaters. Like vaccines, bacteriophage-based products used in humans need to be updated over time—especially when bacteria develop resistance—just as the flu-vaccine cocktail is tailored anew each year. Bacteriophages, however, do not produce active immunity against a specific pathogen as 'regular' vaccines do. Rather, they are antimicrobials, with a secondary competence of boosting the immune system. As such, they are perhaps better considered as similar to therapeutic vaccines.

Sidebar A | Further reading

d'Herelle F (1917) *C R Acad Sci* **165:** 373–375.
In this historical paper, the first isolation of bacteriophages for use in treatment and prophylaxis of infectious diseases is described.

Kutateladze M, Adamia R (2010) *Trends Biotechnol* **28:** 591–595.
The authors report on the growing body of literature describing the validation of the use of bacteriophages for therapy and prophylaxis in the war against drug-resistant bacteria.

Pirnay JP *et al* (2011) *Pharm Res* **28:** 934–937.
The authors stress the importance of a *sur-mesure* approach for phage therapy.

Merabishvili M *et al* (2009) *PLoS ONE* **4:** 1–10.
A quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials is described.

Therapeutic vaccines fall under a third category of human medicinal products, the advanced-therapy medicinal products (ATMPs). ATMPs are defined in directive 2001/83/EC as complex therapeutic products for gene therapy, cell therapy or tissue regeneration, and have their own regulatory framework. Obviously, though, natural bacteriophages are not somatic cells or tissue-engineered medicinal products and are not natural products used in gene therapy, since they are not genetically modified.

The conclusion from the arguments presented above is that phage therapies should probably be classified as biological human medicinal products, despite the poor fit with this classification. Phage therapies do not fit into a single category perfectly, but this choice would be in accordance with the current UK practice of classifying bacteriophages.

If bacteriophages are regarded as human biological medicinal products, they must adhere to the relevant legal framework: any therapy has to demonstrate safety and efficacy and conform to quality standards. Bacteriophages exist ubiquitously in the environment, including in the human body. They specifically infect certain bacteria, but do not attack other bacterial strains or eukaryotic human cells. To assume that bacteriophages will be safe for therapeutic use and ought not to require extensive studies that would delay their clinical use, therefore, seems appropriate. Even so, clinicians should prospectively collect and register data and clinical outcomes of phage therapies to create a body of information for further research and on which applications can build.

In regard to efficacy and safety, only virulent, exclusively lytic phages are generally considered to be clinically useful because they kill their host cells and do not integrate into the bacterial host genome. Therefore, the presence of temperate bacteriophages must be strictly excluded. Detailed molecular characterization of the bacteriophage genome is also mandatory to exclude the presence of any toxin genes or antibiotic-resistance genes.

With respect to quality, a combination of physical, chemical and biological tests could be used to characterize bacteriophage-based products, together with standard quality control procedures applied to the production process. Bacteriophages should be produced in a non-pathogenic bacterial host and the final therapeutic preparations must be pure (absent of residual contaminating bacteriophages and other host cells), sterile, apyrogenic and pH neutral [3]. Such a focused approach to guarantee safety, quality and efficacy could enable clinicians to quickly prepare phage therapeutics against severe infections with multidrug-resistant pathogens.

The current regulatory regime for human biological medicinal products, which implies the conduct of clinical trials and the submission of a full product dossier compliant with directive 2001/83/EG, imposes expensive and time-consuming overheads on the urgent development of phage therapies. If we consider the example of a patient currently infected with a multidrug-resistant bacterial strain who needs immediate treatment because antibiotics have failed, the need to conduct clinical trials and compile dossiers is not feasible within the time frame required to develop a targeted *ad hoc* therapy, and would not allow timely treatment of the patient. Isolation of a bacteriophage to combat the infection, preparation of a therapeutic dose and its administration to the patient needs to be done within days. Under the human biological medicinal product framework, we would have to wait 8–10 years until clinical studies have demonstrated safety and efficacy.

"...the full therapeutic potential of natural bacteriophages can only really be exploited through a patient-specific approach"

Another problem is the massive cost of conducting clinical studies. Non-profit clinics and research institutes will not be able to shoulder the financial burden of a regulatory regime originally designed for drug development by pharmaceutical companies. Some companies, such as Eli Lilly, have invested in bacteriophage-based products or cocktails for human treatment, but the host-specificity of phage therapies excludes uniform production and clinical application. Large-scale production of natural bacteriophages might be helpful in some instances, such as in cases of epidemic outbreaks or in clinical programmes where phage cocktails are regularly updated, but the full therapeutic potential of natural bacteriophages can only really be exploited through a patient-specific approach.

The most likely places that patient-specific phage therapies would be administered are hospitals, in close collaboration with associated microbiological laboratories that would select and isolate the most suitable bacteriophages. This approach would require a simplified regulatory framework, given that neither time nor money is available. From this perspective, bacteriophage therapy resembles the historical context of ATMPs, which were mainly developed at clinics and academic research institutions and were only recently brought under the human medicinal product legislation (regulation 1394/2007). The legislation exempts hospitals from the regulatory framework if a cell therapy is applied under the direct supervision and prescription of a medical doctor for a specific patient (article 28 of regulation [EC] No. 1394/2007), but for ATMPs, national rules apply instead.

As argued above, bacteriophages should probably be classified as human medicinal products. Unfortunately, directive 2001/83/EG does not provide a hospital exemption for these. It does state, however, that it "shall not apply to any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient (commonly known as the magisterial formula)". Even if this clause allowed hospitals to bypass the costly requirement to demonstrate safety and efficacy, hospital pharmacists can only use licensed products as components for magisterial preparations. Since natural bacteriophages are not licensed products, this regulatory bypass would be difficult to implement.

How then can the regulatory framework be adapted to allow hospitals to design and administer tailor-made phage therapies? Although regulators are responsible for applying regulations, a regulation itself can only be changed through legislative action. We suggest an adapted regulatory framework, inspired by the existing legislation governing ATMPs, which includes exemption for the hospital-based use of cell and gene products and therapies. Hospitals should be granted exemption for biological human medicinal products, accompanied by specific regulation for phage therapies developed from natural bacteriophages with regard to safety, potency, purity and toxicity. Pharmaceutical companies developing

products based on natural bacteriophages would still have to abide by the normal regulations that apply to biological medicinal products. Thus, such a regulatory framework would distinguish between the hospital-based (tailor-made) use of natural bacteriophages in patients and the industrial production and distribution of uniform phage products. Quality and safety criteria would be specified and efficacy documentation required, but it would allow treating physicians to fully exploit the coevolutionary aspects of natural bacteriophages for the benefit of patients (Sidebar A).

It is necessary to start talking to regulators and legislators and persuade them of the prudence of a dedicated legal framework for bacteriophage therapy. Doing nothing to address the growing bacterial resistance to antibiotics is not an option. Considering that more than 20,000 European citizens die annually from untreatable bacterial infections, Europe and its member states should find the courage and creativity to financially, technically and legally support the introduction of phage therapies throughout Europe.

ACKNOWLEDGEMENTS

The authors I. Huys, J.-P. Pirnay, R. Lavigne, D. De Vos and G. Verbeken are members of the 'Phagebiotics' research community, supported by the FWO Vlaanderen.

CONFLICT OF INTEREST

I.H., J.-P.P., R.L., D.D.V., S.J. and G.V. are members of the non-profit organization 'Phages for Human Application Group Europe (P.H.A.G.E.)'.

REFERENCES

1. Bush K *et al* (2011) Tackling antibiotic resistance. *Nat Rev Microbiol* **9:** 894–896
2. Roberts R *et al* (2009) Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. *Clin Infect Dis* **49:** 1175–1184
3. Barr JJ *et al* (2013) Bacteriophage adhering to mucus provide a non-host-derived immunity. *Proc Natl Acad Sci USA* **110:** 10771–10776

Isabelle Huys, Minne Casteels and Gilbert Verbeken are at the Department of Pharmaceutical and Pharmacological Sciences, KU Leuven in Leuven, Belgium.

Jean-Paul Pirnay, Daniel De Vos, Gilbert Verbeken and Serge Jennes are at the Burn Wound Centre, Queen Astrid Military Hospital in Brussels, Belgium.

Rob Lavigne is at the Laboratory of Gene Technology, KU Leuven, Belgium.

E-mail: isabelle.huys@ kuleuven.be

EMBO *reports* (2013) **14,** 951–954; published online 18 October 2013; doi:10.1038/embor.2013.163

4 Comparison of the bacteriophage therapy concept with other medicinal products and assessing the implementation of the bacteriophage therapy concept with regulatory agencies

4.1 Comparison with the traditional medicinal products (Study 5) and insights from national and European agencies (Study 6)

4.2 Comparison to the historical development of Advanced Therapy Medicinal Products (Study 7)

4.1 Comparison with the traditional medicinal products (Study 5) and insights from national and European agencies (Study 6)

4.1.1 Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine

G. Verbeken, J.P. Pirnay, D. De Vos, S. Jennes, M. Zizi, R. Lavigne, M. Casteels, I. Huys

Arch Immunol Ther Exp. 2012; DOI 10.1007/s00005-012-0175-0

International scientific journal, peer-reviewed

4.1.1 Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine

G. Verbeken, J.P. Pirnay, D. De Vos, S. Jennes, M. Zizi, R. Lavigne, M. Casteels, I. Huys

Arch Immunol Ther Exp. 2012; DOI 10.1007/s00005-012-0175-0

International scientific journal, peer-reviewed

Arch. Immunol. Ther. Exp.
DOI 10.1007/s00005-012-0175-0

REVIEW

Optimizing the European Regulatory Framework for Sustainable Bacteriophage Therapy in Human Medicine

Gilbert Verbeken · Jean-Paul Pirnay · Daniel De Vos · Serge Jennes · Martin Zizi · Rob Lavigne · Minne Casteels · Isabelle Huys

Received: 3 January 2012 / Accepted: 21 February 2012

Abstract For practitioners at hospitals seeking to use natural (not genetically modified, as appearing in nature) bacteriophages for treatment of antibiotic-resistant bacterial infections (bacteriophage therapy), Europe's current regulatory framework for medicinal products hinders more than it facilitates. Although many experts consider bacteriophage therapy to be a promising complementary (or alternative) treatment to antibiotic therapy, no bacteriophage-specific framework for documentation exists to date. Decades worth of historical clinical data on bacteriophage therapy (from Eastern Europe, particularly Poland, and the former Soviet republics, particularly Georgia and Russia, as well as from today's 27 EU member states and the US) have not been taken into account by European regulators because these data have not been validated under current Western regulatory standards. Consequently, applicants carrying out standard clinical trials on bacteriophages in Europe are obliged to initiate clinical work from scratch. This paper argues for a reduced documentation threshold for Phase 1 clinical trials of bacteriophages and maintains that bacteriophages should not be categorized as classical medicinal products for at least two reasons: (1) such a categorization is scientifically inappropriate for this specific therapy and (2) such a categorization limits the marketing authorization process to industry, the only stakeholder with sufficient financial resources to prepare a complete dossier for the competent authorities. This paper reflects on the current regulatory framework for medicines in Europe and assesses possible regulatory pathways for the (re-)introduction of bacteriophage therapy in a way that maintains its effectiveness and safety as well as its inherent characteristics of sustainability and in situ self-amplification and limitation.

Electronic supplementary material The online version of this article (doi:10.1007/s00005-012-0175-0) contains supplementary material, which is available to authorized users.

G. Verbeken (✉) · J.-P. Pirnay · D. De Vos
Laboratory for Molecular and Cellular Technology,
Burn Wound Centre, Queen Astrid Military Hospital,
Bruynstraat 1, Brussels, Belgium
e-mail: gilbert.verbeken@mil.be

G. Verbeken · M. Casteels · I. Huys
Department Pharmaceutical and Pharmacological Sciences,
Centre for Pharmaceutical Care and Pharmacoeconomics,
KU Leuven, Leuven, Belgium

S. Jennes
Burn Wound Centre, Queen Astrid Military Hospital,
Brussels, Belgium

M. Zizi
Department of Physiology, Free University Brussels,
Brussels, Belgium

M. Zizi
Section Health of the Division Well-Being
(Belgian Defense Staff), Queen Astrid Military Hospital,
Brussels, Belgium

R. Lavigne
Laboratory of Gene Technology,
KU Leuven, Leuven, Belgium

I. Huys
Center for Intellectual Property Rights,
KU Leuven, Leuven, Belgium

Published online: 17 April 2012

Keywords Bacteriophage · Therapy · Human · European · Regulatory · Legal

Lack of an Adequate European Regulatory Framework for (Natural) Bacteriophage Therapy

The European Directive 2001/83/EC[1] is considered the main code governing medicinal products for human use within the European Union. This piece of legislation was designed to specify the file requirements for the authorization of classical medicinal products (e.g., small chemical molecules). Specific regulatory aspects were included for some types of medicinal products[2] (see Table 1), such as radiopharmaceuticals, herbal medicinal products, homeopathic medicinal products, biologicals (vaccines, toxins or serums) and advanced therapy medicinal products (ATMPs). Historically, herbal medicinal products are at the origin of a large number of commonly used pharmaceuticals and cannot be documented as, for instance, small chemical medicinal products. For ATMP, a separate regulation (No 1394/2007[3]) was established with specific scientific and procedural features adapted to these complex medicinal products.

This paper deals strictly with bacteriophage therapy based on the use of natural bacteriophages in human therapy. Natural bacteriophage therapy uses bacteriophages that are not genetically modified. Natural bacteriophages have been known for decades to be anti-infectious agents that can be used complementary to, or as an alternative for, antibiotic treatment (Abedon et al. 2011; Caplin 2009; Górski et al. 2009a, b; Housby and Mann 2009; Kutateladze and Adamia 2008; Kutter et al. 2010; Monk et al. 2010; Skurnik and Strauch 2006; Sulakvelidze et al. 2001; Thiel 2004). Natural bacteriophages can be isolated from the environment, identified, up-scaled, purified and used as antimicrobial agents (Gill and Hyman 2010; Merabishvili et al. 2009). In view of their potential therapeutic uses, it is imperative that natural bacteriophages be processed according to the appropriate quality and safety standards. However, no such framework detailing the file requirements specific to (natural) bacteriophage therapy products exists today (Shorthose et al. 2010). Therefore, a specific and appropriate quality and safety documentation framework is needed for the application of natural bacteriophages on humans.

[1] http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_83_cons2009/2001_83_cons2009_en.pdf (consolidated version).

[2] http://ec.europa.eu/health/files/eudralex/vol-1/dir_2003_63/dir_2003_63_en.pdf.

[3] http://ec.europa.eu/health/files/eudralex/vol-1/reg_2007_1394/reg_2007_1394_en.pdf.

Table 1 Directive 2001/83/EC of the European Parliament and of the Council of 6 NOV 2001 on the Community Code Relating to Medicinal Products for Human Use (consolidated version)

Annex 1
Analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of medicinal products
Part I: Standardized Marketing Authorization Dossier Requirements
Part II: Specific Marketing Authorization Dossier Requirements
Well-established medicinal use
Essentially similar medicinal products
Additional data required in specific situations
Similar biological medicinal products
Fixed combination medicinal products
Documentation for applications in exceptional circumstances
Mixed marketing authorization applications
Part III: Particular medicinal products
Biological medicinal products
Plasma-derived medicinal products
Vaccines
Radio-pharmaceuticals and precursors
Radio-pharmaceuticals
Radio-pharmaceutical precursors for radio-labelling purposes
Homeopathic medicinal products
Herbal medicinal products
Orphan medicinal products
Part IV: Advanced therapy medicinal products
Gene therapy medicinal products
Somatic cell therapy medicinal products
Tissue engineered products

http://ec.europa.eu/health/files/eudralex/vol1/dir_2001_83_cons2009/2001_83_cons2009_en.pdf

http://ec.europa.eu/health/files/eudralex/vol-1/dir_2003_63/dir_2003_63_en.pdf

In this paper, we investigate whether bacteriophage therapy can be situated within existing European regulatory frameworks. We then summarize possible regulatory pathways at different policy levels. Finally, we reflect on several alternative approaches.

Can Natural Bacteriophages Used in Anti-Microbial Therapy on Humans be Situated Within One of the Existing Particular or Specific European Legislation Frameworks Governing Medicinal Products?

Bacteriophages are not comparable to standardized chemical medicinal products (Abedon and Thomas-Abedon 2010; Krylov 2011, Payne and Jansen 2003; Pirnay et al. 2011; Ryan et al. 2011). However, it is worthwhile to call to attention a number of particular and specific regulatory

frameworks in European legislation governing medicinal products and analyze briefly where bacteriophages may or may not fit in (see Table 1).

Biologicals

Biologicals are products whose active substance is biological. At first sight and in view of the nature of several approved biologicals, the "biological medicinal product" chapter of the consolidated 2001/83 EC Directive could be considered applicable to bacteriophages given the biological nature of natural bacteriophage products. That is, the active substance of bacteriophage therapy seems to have a biological origin. However, only a few types of biological medicinal products, such as plasma-derived medicinal products and vaccines, have a dedicated specific documentation package defined under the current biological medicinal products legislation. Bacteriophages, thus, probably would not qualify as biologicals.

Homeopathic Products

Products based on homeopathy are also regulated through a specific category under the European consolidated 2001/83 EC Directive. It may seem surprising that these homeopathic "products" can be documented as medicinal products given the difficulty applicants have with proving their products' (even limited) health claims to the competent authorities. Bacteriophage-based therapeutic products are not comparable to homeopathic products. Bacteriophages have the potential to kill bacteria and have a clear, specific antibacterial effect (Bush et al. 2011; Fernebro 2011; Maura and Debarbieux 2011).

Advanced Therapy Medicinal Products

ATMPs are characterized as complex high-tech therapeutic products with technical specificities that require precise legal definitions. The ATMP section of the consolidated 2001/83 EC Directive only covers products for gene therapy, somatic cell therapy and tissue engineered products. In this sense, one could argue that natural bacteriophages are not ATMPs since their nature differs significantly from that of the gene and cell-based therapeutics currently covered by this regulation. Isolation and production technology used for natural therapeutic bacteriophages do not have the same technological complexity as that used for ATMPs, although one could argue that it is also a complex process (e.g., Matinkhoo et al. 2011; Puapermpoonsiri et al. 2010). This fact does not, however, mean that natural therapeutic bacteriophages are the same as ATMPs.

Well-Established Medicinal Products

European legislation considers a medicinal product to be "well established" if data confirm that the product has been used systematically within the European Community for more than a decade. In addition, the available documentation should assess safety and effectiveness and must include an overview of relevant scientific literature. Based on this criteria, one could potentially classify natural therapeutic bacteriophages as "well-established medicinal products" because they were in use long before the European Medicinal Product Regulation came into force and as far back as the early 1920s (Abedon et al. 2011; Bruynoghe and Maisin 1921; Górski et al. 2009a, b; Housby and Mann 2009; Kropinski 2006; Kutter et al. 2010). Regulators could counter, however, that most (but not all) clinical evaluation dealing with bacteriophage therapy was compiled outside the current 27 EU member states (Fortuna et al. 2008; Górski et al. 2009a, b; Khawaldeh et al. 2011; Kutateladze and Adamia 2010; Rhoads et al. 2009; Wright et al. 2009) and that the proven positive clinical effects were not documented by state-of-the-art, Western-standard-controlled clinical trials.[4] For these reasons, this "well-established medicinal product" framework may not entirely apply to bacteriophage therapy either.

Regulatory Pathways for Bacteriophage Therapy as Discussed at Different Policy Levels

The lack of adequate regulatory guidance for documenting safety, quality and effectiveness of natural bacteriophages when used as therapeutics prompted a first attempt to clarify the situation at the European level [European Parliament and European Medicines Agency (EMA)], followed by discussions at the national level.

European Parliament

On 14 February 2011, a Parliamentary Question[5] was sent to the cabinets of two Members of the European Parliament [Ivo Belet (Belgium/PPE) and Catherine Trautmann (France/S&D)] who addressed the Question to the European Commission and Council. Belet and Trautmann began by explaining to the Commission what "bacteriophage therapy" was. Second, they explained what the existing regulatory hurdles are when working with natural

[4] http://www.eortc.be/services/doc/clinical-eu-directive-04-april-01.pdf.

[5] http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+WQ+E-2011-001144+0+DOC+XML+V0//EN&language=CS.

therapeutic bacteriophages in Europe (Pirnay et al. 2011; Verbeken et al. 2007). Then they raised the question of how bacteriophage therapy is currently regulated in Europe and whether the Commission would consider creating an extra documentation box, "Bacteriophage Therapy", under the "particular" European Medicinal Product framework.

On 29 March 2011, Mr. Dalli, the European Commissioner for Health and Consumer Policy, formulated his written answer on behalf of the European Commission[6]: "The EU's legislation on medicinal products does not define specific requirements related to bacteriophage therapy or medicines composed of bacteriophages". In addition, his answer goes on to say, "the Commission considers that the existing regulatory framework as explained above is adequate for bacteriophage therapy without the need for an extra set of documentation for bacteriophage therapy" (see Supplementary Materials Online Resource 1).

European Medicines Agency Innovation Task Force

The European Commission's, thus, did not clarify how the existing regulatory framework (which it characterized as "adequate") should be interpreted in view of bacteriophage therapy. Clarification was sought at the level of the EMAs Innovation Task Force (ITF). The ITF is tasked with facilitating the informal exchange of information and providing guidance in the early stage of medicinal product development (see Box 1 of Appendix). A request for a Briefing Meeting was submitted to the ITF, supported by specific briefing documents (Merabishvili et al. 2009; Pirnay et al. 2011; Verbeken et al. 2007). The objectives of the meeting were to clarify bacteriophage therapy's classification, to identify the type of documentation needed to launch clinical trials testing natural therapeutic bacteriophages and/or to get marketing authorization and, finally, to discuss a two-way regulatory route for bacteriophage therapy, as described by the author(s) (Pirnay et al. 2011). In short, the "two-way regulatory approach" differentiates between the industrial development of uniform bacteriophage products meant for uniform market placement[7] and the tailor-made production of patient-specific natural therapeutic bacteriophages.

On July 12, the authors' delegation (6 experts, hereafter called "the delegates") met with the ITF (13 experts) and discussed the current situation and certain specific aspects. Subsequently, the final opinion was published by the ITF in the Briefing Meeting Report,[8] reflecting the opinions of the members of the ITF and of the contributing experts. These opinions should be interpreted as a preliminary set of scientific considerations related to the information presented.

Point of View of the Delegates

Working with natural bacteriophages in a non-profit hospital environment contrasts with industrial approaches to the preparation of bacteriophage-based products in that the latter prioritizes regular market authorization while the former does not. Bacteriophage therapy should be understood as a "therapy concept". Bacteriophages and bacteria co-evolve in a dynamic system. Taking this reality into account is crucial if the full sustainable potential of bacteriophages in therapeutics is to be actualized. Industry actors may pursue uniform market placement of bacteriophage cocktails for economic reasons, but this diverges from the idea and practical aspects of bacteriophage therapy itself, which is based first and foremost on a tailor-made patient approach. Additionally, securing intellectual property (IP) protection for tailor-made natural bacteriophages, while likely possible, is far from simple. Phages are widespread and the therapy has existed for years. One can thus anticipate broad patent claims covering production processes, intended (new) uses and the phages themselves. However, such broad patent claims are weak and easy to circumvent. Weak IP protection makes the task of attracting venture capitalists difficult, which means developers of tailor-made natural bacteriophage therapies often lack the financial resources necessary to pursue costly regulatory pathways. The "reusing" or "refitting" of [non-good manufacturing practices (GMP) but carefully developed and delineated] production process developed in the authors' previous study (see Box 2 of Appendix) (Kutter et al. 2010) raises an important regulatory question. Do production processes approved for similar products satisfy EMA regulators and adhere to the necessary safety and quality standards (Merabishvili et al. 2009)?

Final Opinion of the ITF

Medicinal Products

Since the pathway for developing a medicinal product is specific to each indication and each product, the ITF advises applicants to submit, free of charge, a request to have EMA scientific services investigate a specific proposed bacteriophage product's "eligibility" to be classified

[6] http://www.europarl.europa.eu/sides/getAllAnswers.do?reference=E-2011-001144&language=CS.

[7] http://ec.europa.eu/health/files/eudralex/vol-1/reg_2004_726_cons/reg_2004_726_cons_en.pdf.

[8] ITF's Opinion, Briefing Meeting Report, EMA/642573/2011, 22 July 2011, available on request to the author of correspondence.

as a medicinal product.[9] The EMA then evaluates whether that product is a medicinal product based on the description of the active substance, the manufacturing process, the production process, the mechanism of action and the intended clinical application. The European Commission is consulted during that process. At the moment of submission, a product-specific website with a description of the information to be submitted is created, the information is then submitted by the applicant and the EMA consult its experts.

Biologicals or ATMPs

If bacteriophage-based products are considered medicinal products, they will likely be classified as biological medicinal products. In general, a bacteriophage-based product is not considered an ATMP in light of the current, highly specialized ATMP Regulation. This holds unless the Scientific Committee changes its opinion and the European Commission interprets the legislation to include a particular bacteriophage-based product. The ITF does not anticipate this, but this does not preempt the decision (should there be one) made by the Commission and the Scientific Committee.

Dossier Requirements

The specific regulatory requirements necessary for bacteriophage therapy approval are evaluated on a case-by-case basis and are determined by the specific characteristics of the product and its intended use and hence not on the product as such. The quality and safety data presented in the authors' study (Merabishvili et al. 2009; Kutter et al. 2010) were considered to be basic. However, these data could be part of an Investigational Medicinal Product Dossier (IMPD) when launching clinical trials. Additionally, an EMA's Scientific Advise (SA) procedure could also be requested to seek advice on medicinal product development. All information related to SA requests is also available on the EMA website.[10]

It is notable that the EMA explicitly acknowledges efforts to develop tailor-made bacteriophage products. But SA's are still needed for each particular bacteriophage-based product based on a review of the full package submitted in a separate SA procedure. Each natural bacteriophage-based product must prove to be safe, with a suitable dosage and with evidence of clinical significance.

European regulators currently emphasise "Quality by Design", where one designs the critical parameters that form the core of the product before moving forward with development or marketing authorization. One can decide to apply for a product classification (as described above) to get an idea of how the product is positioned and based on this information, can then consider various elements of the product's Marketing Authorization options. One element to consider, for instance, is the production process. The EMA has sufficient insight into what elements may be important for a particular product. It has extensive experience with the manufacturing process, disease frequency and conditions to target (for example for an orphan drug designation), and SA on the quality aspects relevant to these defined elements is also available. The "quality by design" approach could potentially be very useful, but would require some modelling and simulation exercises (especially in view of co-evolutionary aspects). It could nonetheless be particularly critical to defining the key parameters for production processes or manipulations, which could then provide the basis for further development.

Evolving Products

Bacteriophages are not considered unique as "evolving" products. The EMA has handled precedents in this respect, for instance, living cell preparations and the flu vaccine. Every 1–2 years, the recommended strains for the seasonal flu vaccines are reviewed to investigate whether an adaptation is needed to accommodate for the flu strain(s) that will likely appear in the next season. The launch of a new vaccine needs to be supported by facts and data and an appropriate risk management strategy for the product. Likewise, bacteriophage product for a given indication and produced according to a given manufacturing process could also be updated every 1–2 years. While natural bacteriophage therapy is an unprecedented particular case, the EMA has dealt with similar products before, which suggests that it would be able to handle natural bacteriophage therapy as well.

Procedure

Authorization of a medicinal product follows either a centralized, decentralized, or mutual recognition procedure, depending on the product's categorization. For a biological medicinal product, authorization processes are not homogeneous between EU member states, as applicants may opt for a national rather than European procedure and national procedures can differ from country to country. However, the product's quality, safety and efficacy must be

[9] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp&mid=WC0b01ac05800ba1d9&jsenabled=true.

[10] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac05800ba1d9.

demonstrated in all procedures. ATMPs can only be authorized via the centralized procedure.

Personalized Medicine

One possible method for using phages in patient treatment could be to provide a first injection of natural bacteriophages geared toward attacking a broad bacterial infections problem. This would then be followed by a treatment with a specific (personalized) set of bacteriophages, selected and applied to the patient to combat an emerging additional problematic bacterial strain specific to that patient. Tests should be performed before administering the second batch of personalized bacteriophages. The best way to use bacteriophages—and this is where it becomes especially personalized—would be to take the original bacteriophage (to which resistance emerged) and let it evolve (in vitro), in parallel with the patient's infecting strain, that is, to use the bacteria-phage dynamics to actually evolve a phage. Although it complicates matters, it exemplifies the real power of this approach. This approach is considered comparable to some therapies using autologous cells that are manipulated ex vivo and then returned to the patient. There is a particular need for critical dialogue with EMA Scientific Committees on this topic. However, in the case of bacteriophage therapy, the target is not a human cell but a bacterial cell. There are millions of bacteria and phages living in and on the human body. The question thus arises as to whether bacteriophages can be considered "human body material". If so, bacteriophages would be covered by the European Human Cells and Tissue Directive.[11] However, no real answer has been provided on this question.

Although bacteriophages differ from conventional biological products and an adaptation of the current legislation is urgently needed, regulators like the EMA stress that they do not make new rules; they only apply existing regulations. It is the view of the ITF that a new regulation cannot be "invented" by regulators.

Actions Proposed by the ITF

First, a request could be submitted to the Committee for Medicinal Products for Human Use to investigate the eligibility of a particular phage-based product.[12] If bacteriophage therapy could be used for an orphan indication, an application for Orphan Designation could be considered, making use of specific incentives in the law such as protocol assistance and fee reductions. Second, the option remains to apply for EMA scientific advice to delineate a clinical development strategy as well as specific protocols and models of how to prepare the product and the precise use of bacteriophages, for instance, whether to use a fixed cocktail or a fixed cocktail complemented with personalized bacteriophages. Overall, the clinical development of bacteriophage therapy needs to show that phage products are safe (Phase I), that the proposed dose will be the most effective one (Phase II) and that in clinical trials (in which bacteriophages are applied exactly as they will be used in practice) the therapy shows efficiency, taking into account the "appropriate indication" and "clinical end point" (Phase III).

National Competent Authority

Following the opinion of the ITF at EMA, a discussion took place with the Belgian Competent Authority, the Belgian Federal Agency for Medicines and Health Products (FAMHP), which, though unofficially, expressed the following view:

Medicinal Products

In line with EMA, natural bacteriophages used as therapeutics are considered medicinal products, based on their nature and intended use.

Biologicals or ATMPs

In addition, bacteriophages are seen as biological medicinal products that can be commercialized via a national regulatory procedure. However, these natural bacteriophages are not considered as ATMPs. This was the conclusion reached at a consultation by FAMHP of EMA's Committee for Advanced Therapies. This standpoint has specific consequences for hospital-based therapies. The ATMP Regulation as such is a *lex specialis* which introduces additional provisions to those laid down in Directive 2001/83/EC. The scope of the ATMP Regulation is to regulate the authorization of ATMPs intended to be placed on the market in European Union member states, either prepared industrially or manufactured by a method involving an industrial process, in accordance with Directive 2001/83/EC. ATMPs are excluded from the scope of the European ATMP Regulation if they are prepared on a non-routine basis according to specific quality standards and used within the same member state in a hospital under the exclusive professional responsibility of a medical practitioner and an individual medical prescription for a custom-made product for an individual patient. This

[11] Directive 2004/23/EC of the European parliament and of the council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.

[12] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp&mid=WC0b01ac05800ba1d9&jsenabled=true.

exclusion is called the hospital exemption. The precise interpretation of the definition of hospital exemption is left to the National Competent Authorities. For instance, the (national) definition of "non-routine use" can be different in each member state. However, the member states have to ensure that relevant community rules related to quality and safety are not undermined. This national procedural pathway for hospital exemptions could be of interest to hospitals working with their own tailor-made natural bacteriophage therapies. However, since the ATMP framework is not considered to be relevant by the EMA and Belgian regulators when positioning natural bacteriophages, the hospital exemption procedure cannot be applied to bacteriophage therapy and therefore hospitals interested in bacteriophage therapy cannot benefit from this hospital exemption.

File Requirements

At the national level (FAMHP), the possibility of obtaining an "early phase" clinical trial status for bacteriophage therapy could be considered. It should be pointed out that, at present, obtaining an authorization to produce IMP's (Trouet et al. 2007) may be an impossible burden for hospitals. It is even questionable as to whether a full authorization is always needed for the limited IMP production that is required for a phase I clinical trial, for instance. At this level, as at the European level, there is a need for a pragmatic approach.[13]

Actions Suggested by the National Competent Authority

A reduced IMPD could be submitted for evaluation, and interactions with the National Competent Authority concerning the content of the application could be initiated in a constructive and open-minded way.

Reflection by the Authors

Theoretically, the proposal to opt for a full market authorization via a simplified procedure at the National Competent Authority appears feasible. However, this proposed approach lacks an awareness of the specific "personal medicinal character" of natural bacteriophages and reflects only a (classical) medicinal product development approach. Today, there is no legal definition for "Personalized Medicine" and most preparations intended for use as "personalized medicinal products" are not really "personal", meaning that they are produced for a very small patient population and not for one patient as such.

Hospitals willing to use bacteriophage therapy in clinical practice, working independently from industry in a non-profit setting and using tailor-made phage products to treat patients, are not necessarily interested in obtaining marketing authorization and IP protection. Moreover, a stringent marketing authorization approach, even with simplified procedures, is nearly impossible for hospitals to manage to date. In addition, in view of the enormous challenges related to rapidly progressing bacterial resistance, a product development process that takes many years does not seem to be the best way forward.

Belgian and UK Notified Bodies

Given the opinions expressed by European and national regulators and given that uncertainties remain regarding the appropriate regulatory pathway for a natural bacteriophage-based product, it is worth considering whether such a product could fall under the Medical Device framework. To find out whether such an approach would work for bacteriophages, a burn wound ointment that is actually on the market as a medical device was supplemented with natural bacteriophages. In fact, the antimicrobial component of the burn wound ointment (in this case an enzyme) was replaced with natural bacteriophages.

In May 2011, a request for opinion was submitted to a Belgian Notified Body (SGS) authorized to certify medical devices for market placement. SGS Belgium forwarded the question to their London office, and this SGS London office contacted the UK Competent Authority, the Medicines and Healthcare Products Regulatory Agency.

Medical Devices Framework and Current Applications

Directive 2001/83/EC defines a medicinal product as "any substance or combination of substances presented as having properties for treating or preventing disease in human beings or any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis". According to the Medical Devices Directive 93/42/EC,[14] medical devices support the restoration, correction or modification of physiological functions as described above, rather than being the cause of it. Some medical device manufacturers drop the primary therapeutic claim for the proposed product so that this product can be covered by the Medical Devices Directive 93/42/EC. In light of the above, it seems useful to consider whether bacteriophage therapy

[13] http://www.pharma.be/assets/files/2309/2309_1295209549827530 81.pdf?bcsi_scan_3c79e7817cdc4fd7=0&bcsi_scan_filename=2309_ 129520954982753081.pdf.

[14] Council directive 93/42/EEC of 14 June 1993 concerning medical devices (consolidated version).

can be covered by the Medical Device Directive. Under current practice, for instance, as long as a burn wound ointment only moistens and protects a wound bed (primary claims), these ointments are considered to be medical devices, even when they contain antimicrobials that keep the ointment aseptic.

Opinion of the UK Notified Body

Adding natural bacteriophages to a burn wound ointment would turn a medical device (such as the ointment described above) into a medicinal product, based on the fact that phages have a "targeted action".

Reflection by the Authors

It is true that phages have a targeted action when considering a tailor-made use of bacteriophages for treating specific infections on defined patients. However, in theory, uniform bacteriophage cocktails could be placed on the market as medical devices, without claiming any primary therapeutic effects, circumventing the current and usual medicinal product regulation. This cannot be the intention of regulators. Therefore, the preferred route is the creation of an adequate regulatory framework for therapeutic natural bacteriophages.

Alternative Regulatory Approaches

During the consultations at different regulatory levels, certain specific concepts and types of product status were mentioned that are useful to investigate in greater detail.

Medicinal (Whole) "Animal" Product Status

It is worthwhile to consider whether bacteriophages can be regarded as "whole animals" (as mentioned in Art. 1 of the consolidated Directive 2001/83/EC), such as larvae (medicinal maggots used, for instance, in debridement of wounds) and leaches (medicinal leaches used, for instance, in the stimulation of vascularization after amputation), all of which are present on the European market for therapeutic use. Some of these types of products are regulated via the current European medicinal products regulation. Product files have been compiled and licenses have been granted in that area, and in some cases, such "animals" have even been GMP produced. In practice, some manufacturers of these "whole animal" products try to avoid the full GMP requirement in a way similar to that described above in the medical device section. For instance, manufacturers put their animals in a dressing and call this dressing a "biosurgical wound dressing". The animals (e.g., maggots) are "aseptically cultivated/raised". Preparations are available by prescription only and are individually produced for a specific patient.[15] Indeed, such a situation is preferable and compatible with bacteriophage-based therapy. However, defining a natural bacteriophage as a "whole animal" is somewhat far-fetched.

Magisterial (Compounded) Preparations

During the GEEPhage 2011 meeting (March 2011),[16] the French Competent Authority explained that the pathway that regulates magisterial hospital preparations could perhaps provide a regulatory gateway for hospitals that have the intention to use, in-house, home-made preparations of natural bacteriophages on a non-routine basis and in a patient-tailored manner.

Under Belgian legislation, certain specific considerations need to be taken into account. First, if the bacteriophage cocktail is not authorized to enter the European market, a "product monograph" must be compiled. Specifically, this means that a documentation and description of the identity, the purity and the properties of the natural bacteriophage preparation must be provided. This monograph must be submitted for evaluation to the Belgian Pharmacopeia Commission. The bacteriophage preparation as published by Merabishvili et al. (2009) is considered as "crude material" by regulators. To be considered as a magisterial preparation, the preparation must be produced under GMP. After a positive answer from the Pharmacopeia Commission, the microbiological lab of the particular hospital can be licensed by the necessary Belgian Minister as a supplier of "crude materials". Only then may a pharmacist at the respective hospital use that particular bacteriophage cocktail as a component for magisterial preparations. It is clear that this pathway is not adapted to hospitals that do not have the ambition, intention, or financial resources to place a product on the national or European market.

Medicinal Products without Marketing Authorization: "Compassionate Use"

Medicinal products that are not (yet) authorized to enter the European market can be used on patients when this use is covered by compassionate use programmes, regulated at the national level. Compassionate use programmes are meant for patients with chronically or seriously debilitating diseases or for patients whose disease is not considered to be treated satisfactorily with an authorized product (Dodds-Smith and Valverde 2009). As tailored bacteriophage

[15] http://www.biomonde.de/English/protected/bio_produkt.html.

[16] http://geephage.org/bacteries/.

cocktails (Merabishvili et al. 2009) are in fact not in the pipeline to obtain European marketing authorization, the compassionate use pathway seems untenable yet still possible. On the other hand, compassionate use programmes would definitely be relevant to potential uniform bacteriophage preparations that are currently involved in marketing authorization procedures.

The Declaration of Helsinki is particularly relevant for medicinal products not intended for placement in the European market under license. Paragraph 35 of the Declaration of Helsinki[17] states the following: "In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health, or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available".

Taking this paragraph into account, natural tailored bacteriophages can be used on patients in need when the Helsinki conditions are fulfilled. This is what is sporadically done in Europe at the moment, for instance, at the Queen Astrid Military Hospital in Brussels, Belgium. Due to its "exceptional" character, the Helsinki procedure is not a regulatory procedure with the potential to introduce bacteriophage therapy into modern medicine in any widespread, sustainable sense.

National Legislation Defining Exemptions

Some countries have published national legislation to regulate certain specific products or techniques made available in the country in a non-profit setting. In Poland, phage therapy is considered an experimental treatment. Experimental treatment occurs when a physician introduces new or only partially tested diagnostic, therapeutic, or prophylactic methods for the direct benefit of the person being treated. In contrast, an investigational experiment has the primary purpose of broadening medical knowledge. Two basic items are prerequisites for experimental therapy in Poland: (a) the written informed consent of the patient and (b) approval by an institutional review board (bioethics commission). Furthermore, experimental therapy may only be applied by a qualified doctor and when other available treatment has failed.[18] In Poland, this type of treatment is also covered by the Helsinki Declaration (Kutter et al. 2010; Letkiewicz et al. 2010).

In the Czech Republic there is an (reimbursed) anti-staphylococcal bacteriophage product (lysate) on the market under the trade name Stafal.[19] Stafal was approved for market placement by the Czech National Competent Authority, the State Institute for Drug Control. The product is an anti-staphylococcal phage lysate intended for topical use (registration number 59/0149/89-CS).

When considering clinical trials, the type of exemptions listed above may favour small (to very small) investigator-driven trials, allowing clinicians to conduct pilot studies without the full burden of commercial regulation. However, it is uncertain as to whether bacteriophage therapy would be covered by such exemptions, and even so, bacteriophage therapy would not be covered in a harmonized manner across Europe. Again, there is a need for an adapted framework for defining (natural) bacteriophage therapy at the European level. Member states are forced to try to find solutions at the national level because there is no adequate European framework.

Grey Areas

Aside from bacteriophages, there are some other products that could be called "grey area" products. This means that it is not always clear whether a certain product has to be classified as a medicinal product, as a food supplement (see Box 3 of Appendix), as a cosmetic, as a biocide, as a nutrient, or as a product for regular consumption. The regulatory pathway for each of these categories differs considerably.

In a sense, a natural bacteriophage preparation could also be considered a "grey area" product. As yet, it is unclear whether a bacteriophage-based preparation is really a medicinal product. Some member states have installed a specific commission at their national regulatory authority to tackle "grey area" products. For instance, in Belgium, a "Mixed Commission" is in place that decides on the classification (or not) of a particular "grey area" product as a medicinal product. When the commission decides that a specific product is a medicinal product, the medicinal product regulatory framework (Directive 2001/83/EC) has to be applied. Thus, for "grey area" products, the national competent authorities at the member state level decide whether such a product is a medicinal product, after consulting other relevant stakeholders. They can take European precedents into account and they can, of course, also consult the European Competent Authorities.

If this Mixed Commission were to decide that tailored natural bacteriophages are indeed medicinal products, the question arises as to how Directive 2001/83/EC

[17] http://irb.sinica.edu.tw/declaration%20of%20helsinki%20(2008).pdf.

[18] arts. 29/1, 21/2, and 21/3 of the Polish law on the physician's profession.

[19] http://www.sevapharma.cz/file/Stafal_EN.pdf.

(Consolidated) would then be applied, taking into account its scope. Indeed, Article 2 of the Directive explains that the rules shall only apply to "medicinal products for human use intended to be placed on the market in the European Member States and are either prepared industrially or manufactured by a method involving an industrial process". "In cases of doubt", the Directive mentions, "where, taking into account all its characteristics, a product may fall within the definition of a "medicinal product" and the definition of a product covered by other Community legislation, the provisions of Directive 2001/83/EC shall apply". In addition, the Directive shall also apply to medicinal products intended only for export and to intermediate products. In view of these wordings, "home-made" and "in-house-applied" natural bacteriophage cocktails such as bacteriophage preparations do not seem to be captured by the scope of the Medicinal Products Directive, since such cocktails are not intended to be placed on the market in the European member states. Indeed, everything depends on what the legislator takes "placing on the market" to mean. Informal contacts with EMA and the Belgian National Competent Authority determined that even the in-house application on one patient of an in-house-prepared product could be considered a market placement. However, to date no legal argumentation for this position has been made available, and no such interpretation seems likely to be implemented, especially if this service is not publicly advertised. Nonetheless, it is important that the issue be clarified.

Conclusions

The authors are convinced that products for bacteriophage therapy deserve their own regulatory framework in Europe. Various pharmaceutical products different from the classical chemical molecules already have a proper framework designed under the Medicinal Products Directive 2001/83/EC (see Table 1). Natural bacteriophages are products that proved to have a well-established medicinal use in Eastern Europe and the former Soviet republics. They could be considered a type of Particular Medicinal Products, to be defined under a separate (dedicated) chapter in Annex 1 of the Directive. Alternatively, the optimal framework could be one dedicated specifically to bacteriophage therapy, alongside the present Medicinal Products Directive 2001/83/EC. As regulatory frameworks for other medicinal products have been introduced via particular Directives in the past, a new Directive for bacteriophage therapy could be a suitable solution. This new Bacteriophage Directive could then be called "Directive concerning the Therapeutic use of Natural Bacteriophages". This "new" adapted regulatory framework should certainly also include adequate timeframes, since the actual timeline for a classical drug approval is of the order of months/years/decades while the development of a "new" natural bacteriophage product can happen in a matter of days/weeks. Regulators at the competent national or European authorities (like EMA or FAMHP in Belgium) understand the potential of bacteriophage therapy. However, they cannot change or adapt any legislation. A collective lobby at the European political level could be an option for securing a vote of the European Parliament to adapt the actual European medicinal product regulatory framework. At the international level, dedicated bacteriophage therapy symposia such as the recent Phage 2011 meeting[20] are important at the scientific and technical-industrial level, but additional encounters and meetings are needed as platforms for increasing awareness at the political as well as at the public opinion levels. Classical pharma-symposia should start to integrate bacteriophage therapy issues into their programmes. For this reason, initiatives led by non-profit organizations like GEEPhage, P.H.A.G.E.[21] and PHAGESPOIRS[22] can play an active and primary role for change, particularly because these organizations were created to support bacteriophage research and therapy to the benefit of all patients. As foci of patient support efforts, these organizations play an important role in the creation of greater awareness among competent authorities (regulatory implementation) and policymakers (voting regulation).

Acknowledgments The authors would like to thank Professor Carl Ceulemans from the Military Higher Institute of Defence (Chair of Philosophy, Faculty of Social and Military Sciences, Royal Military Academy, Brussels, Belgium) for his honest, coherent and beneficial interactions concerning the re-introduction of phage therapy in routine clinical settings.

Conflict of interest The authors have no potential conflicts of interest directly relevant to the content of this manuscript.

Appendix

Box 1 Innovation Task Force (ITF)

The "Innovation Task Force" of the European Medicines Agency (EMA)[23] is a multidisciplinary group of scientific, regulatory and legal experts set up at the EMA to provide a forum for early dialogue in the form of "briefing

[20] http://www.libpubmedia.co.uk/Conferences/Phages2011/About.htm.

[21] http://www.p-h-a-g-e.org/Home.html.

[22] http://translate.google.be/translate?hl=nl&sl=fr&tl=en&u=http%3A%2F%2Fphagespoirs.unblog.fr%2F.

[23] http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000334.jsp&mid=WC0b01ac05800ba1d9&jsenabled=true

meetings". The scope of the briefing meetings covers regulatory, technical and scientific issues arising from innovative medicines development, new technologies and borderline products. Within 60 days of receipt of a valid request, the ITF arranges free-of-charge briefing meetings to facilitate the informal exchange of information and the provision of guidance early in a development process. The scientific discussions are led by experts from the European Medicines Agency network, working parties and committees, where the best available scientific expertise is represented. Briefing meetings are meant to complement and reinforce existing formal regulatory procedures (e.g., ATMP classification, ATMP certification, designation of orphan medicinal products, etc.).

Box 2 Quality and safety evaluation criteria bacteriophage cocktail BFC1 (Merabishvili et al. 2009)

Characterization of the bacteriophages:

- Determination of the morphotype
- Complete DNA and proteome analysis (confirm absence of lysogeny and toxin genes)
- Testing host bacteria used in production for absence of (lysogenic) phage (mitomycin test)

QC tests performed by qualified accredited laboratories:

- Phage titer (agar overlay method)
- pH (according to EP)
- Cytotoxicity (ISO10993-5)
- Pyrogenicity (10 ml/kg rabbit, according to USP)
- Sterility (according to EP)
- Confirm morphology and activity towards targeted bacteria (transmission electron microscopy)

Box 3 Bacteriophages introduced into the food chain: some reflections

As a regulatory pathway, legislation for food supplements was investigated. In the US, the Food and Drug Administration (FDA) approved the use of Listex natural bacteriophages for the decontamination of foods.[24] Additionally, in the Netherlands and Switzerland anti listeria bacteriophages are in use for food products.[25] Current practice in the US for bacteriophage-based products is that such products are evaluated 'case-by-case' and finally approved (or not) under the US GRAS regulation (Federal Regulation of Substances Generally Recognized As Safe). In the US, foods and drugs are administered by one competent authority, the FDA, which is a federal structure without an equivalent in Europe. In Europe, the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) are two separate structures, probably explaining why it is a bigger step from food to medicinal products in Europe than it is in the US. However, in 2008 the European Commission (DG Health and Consumers) asked the EFSA to provide technical assistance in relation to the use and mode of action of bacteriophages on food of animal origin (Question No EFSA-Q-2008-400[26]). The scientific opinion of the EFSA panel on biological hazards was endorsed on 22 April 2009. The EFSA panel concluded that some bacteriophages, under specific conditions, have been demonstrated to be very effective in the targeted elimination of specific pathogens present on meat, milk and products thereof. The panel, however, could not conclusively find that bacteriophages could protect such products when re-contamination of the decontaminated products occurs. The panel also proposed that a "case-by-case" evaluation of presented phage products is necessary. This European approach is comparable to the US (GRAS) approach, which is in fact a positive evolution.

Surprisingly, however, some (pro-biotic) food-products define health claims in their dossier without being registered as medicinal products (for example, Actimel[27] and Yakult[28]). These types of products, by definition, also contain natural bacteriophages. It is questionable whether these health claims are evidence-based. In any case, the issue is hot in Europe, especially because Europe recently refused to accept some of the health claims related to specific products based on lack of evidence.[29],[30] Recently, an international workshop on the topic was organized in Brussels[31] entitled "how to design studies to prove the claimed health effects of these food products". Obviously, this field is moving quickly as well.

References

Abedon ST, Thomas-Abedon C (2010) Phage therapy pharmacology. Curr Pharm Biotechnol 11:28–47

Abedon ST, Kuhl SJ, Blasdel BG et al (2011) Phage treatment of human infections. Bacteriophage 1:66–85

Bruynoghe R, Maisin J (1921) Essais de thérapeutique au moyen du bacteriophage du Staphyloccoque [therapeutic trials using bacteriophage of *Staphylococcus*]. CR Soc Biol 85:1120–1121

[24] http://www.prnewswire.com/news-releases/fda-extends-gras-approval-listextm-to-all-food-products-57761742.html

[25] http://www.micreosfoodsafety.com/images/USDA-23-2011-1.pdf

[26] http://www.efsa.europa.eu/en/scdocs/doc/1076.pdf.

[27] http://www.efsa.europa.eu/en/efsajournal/pub/1903.htm.

[28] http://www.efsa.europa.eu/en/efsajournal/pub/1860.htm.

[29] http://www.efsa.europa.eu/en/press/news/110728.htm.

[30] http://www.telegraph.co.uk/health/healthnews/8074182/Probiotic-drinks-do-not-aid-health-Europe-says.html.

[31] http://www.healthclaims.eu/

Bush K, Courvalin P, Dantas G et al (2011) Tackling antibiotic resistance. Nat Rev Microbiol 9:894–896

Caplin J (2009) Bacteriophage therapy: old treatment, new focus? Microbiologist 10:20–23

Dodds-Smith I, Valverde S (2009) The supply of unlicensed medicines for individual patient use. In: Griffin JP (ed) The textbook of pharmaceutical medicine, 6th edn. Wiley-Blackwell, West Sussex, pp 331–346

Fernebro J (2011) Fighting bacterial infections-future treatment options. Drug Resist Updat 14:125–139

Fortuna W, Miedzybrodzki R, Weber-Dabrowska B et al (2008) Bacteriophage therapy in children: facts and prospects. Med Sci Monit 14:RA126–RA132

Gill JJ, Hyman P (2010) Phage choice, isolation, and preparation for phage therapy. Curr Pharm Biotechnol 11:2–14

Górski A, Miedzybrodzki R, Borysowski J et al (2009a) Bacteriophage therapy for the treatment of infections. Curr Opin Investig Drugs 10:766–774

Górski A, Targońska M, Borysowski J et al (2009b) The potential of phage therapy in bacterial infections of the eye. Ophthalmologica 223:162–165

Housby JN, Mann NH (2009) Phage therapy. Drug Discov Today 14:536–540

Khawaldeh A, Morales S, Dillon B et al (2011) Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection. J Med Microbiol 60(Pt 11):1697–1700

Kropinski A (2006) Phage therapy—everything old is new again. Can J Infect Dis Med Microbiol 17:297–306

Krylov V (2011) Use of live phages for therapy on a background of co-evolution on bacteria and phages. Int Res J Microbiol 2:315–332

Kutateladze M, Adamia R (2008) Phage therapy experience at the Eliava Institute. Med Mal Infect 38:426–430

Kutateladze M, Adamia R (2010) Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol 28:591–595

Kutter E, De Vos D, Gvasalia G et al (2010) Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 11:69–86

Letkiewicz S, Międzybrodzki R, Kłak M et al (2010) The perspectives of the application of phage therapy in chronic bacterial prostatitis. FEMS Immunol Med Microbiol 60:99–112

Matinkhoo S, Lynch KH, Dennis JJ et al (2011) Spray-dried respirable powders containing bacteriophages for the treatment of pulmonary infections. J Pharm Sci 100:5197–5205

Maura D, Debarbieux L (2011) Bacteriophages as twenty-first century antibacterial tools for food and medicine. Appl Microbiol Biotechnol 90:851–859

Merabishvili M, Pirnay JP, Verbeken G et al (2009) Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 4:e4944

Monk AB, Rees CD, Barrow P et al (2010) Bacteriophage applications: where are we now? Lett Appl Microbiol 51:363–369

Payne RJ, Jansen VA (2003) Pharmacokinetic principles of bacteriophage therapy. Clin Pharmacokinet 42:315–325

Pirnay JP, De Vos D, Verbeken G et al (2011) The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm Res 28:934–937

Puapermpoonsiri U, Ford S, van der Walle CF (2010) Stabilization of bacteriophage during freeze drying. Int J Pharm 389:168–175

Rhoads D, Wolcott R, Kuskowski MA et al (2009) Bacteriophage therapy of venous leg ulcers in humans: results of a phase I safety trial. J Wound Care 18(237–238):240–243

Ryan E, Gorman S, Donelly RF et al (2011) Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy. J Pharm Pharmacol 63:1253–1264

Shorthose S, Bird & Bird LLP (2010) Guide to EU pharmaceutical regulatory law. Wolters Kluwer, Alphen aan den Rijn

Skurnik M, Strauch E (2006) Phage therapy: facts and fiction. Int J Med Microbiol 296:5–14

Sulakvelidze A, Alavidze Z, Morris JG Jr (2001) Bacteriophage therapy. Antimicrob Agents Chemother 45:649–659

Thiel K (2004) Old dogma, new tricks—21st Century phage therapy. Nat Biotechnol 22:31–36

Trouet C, Gobert M et al (2007) Investigational medicinal products. In: Clinical trials in Belgium: the Belgian implementation of the European Clinical Trials Directives. An operational guidance, 2nd edn. Intersentia, Antwerp

Verbeken G, De Vos D, Vaneechoutte M et al (2007) European regulatory conundrum of phage therapy. Future Microbiol 2:485–491

Wright A, Hawkins CH, Anggard EE et al (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin Otolaryngol 34:349–357

4.2 Comparison to the historical development of Advanced Therapy Medicinal Products (Study 7)

4.2.1 Bacteriophage therapy: fast-forward to the past; *Lessons Identified from the Advanced Therapy Regulation*

G. Verbeken, D. De Vos, A. De Conick, D. Roseeuw, E. Kets, A. Vanderkelen, J.P. Draye, T. Rose, S. Jennes, I. Huys, J.P. Pirnay

Submitted to international scientific journal 'Burns', peer-reviewed

4.2.1 Bacteriophage therapy: fast-forward to the past; *Lessons Identified from the Advanced Therapy Regulation*

G. Verbeken, D. De Vos, A. De Conick, D. Roseeuw, E. Kets, A. Vanderkelen, J.P. Draye, T. Rose, S. Jennes, I. Huys, J.P. Pirnay

Submitted to international scientific journal 'Burns', peer-reviewed

BACTERIOPHAGE THERAPY: FAST-FORWARD TO THE PAST

Lessons Identified from the Advanced Therapy Regulation

G. Verbeken [a,e,f], D. De Vos [a,f], A. De Coninck [b], D. Roseeuw [b], E. Kets [c], A. Vanderkelen [a], J.P. Draye [a], T. Rose [a,c], S. Jennes [c], I. Huys [d,e], C. Ceulemans [f], J.P. Pirnay [a]

[a] *Queen Astrid Military Hospital, Burn Wound Centre, Laboratory for Molecular and Cellular Technology, Bruynstraat 1, 1120 Brussels, Belgium*

[b] *Free University Brussels, University Hospital Brussels (UZ Brussel), Department of Dermatology, Laarbeeklaan 101, 1090 Brussels, Belgium*

[c] *Queen Astrid Military Hospital, Burn Wound Centre, Bruynstraat 1, 1120 Brussels, Belgium*

[d] *KU Leuven, Centre for Intellectual Property Rights, Minderbroedersstraat 5, 3000 Leuven, Belgium*

[e] *KU Leuven, Faculty of Pharmaceutical and Pharmacological Sciences, Herestraat 49, 3000 Leuven, Belgium*

[f] *Royal Military Academy, Department of Behavioural Sciences, Renaissancelaan 30, 1000 Brussels, Belgium*

✉ Queen Astrid Military Hospital, Burn Wound Centre, Laboratory for Molecular and Cellular Technology, Bruynstraat 1, 1120 Brussels, Belgium

Office telephone number: +32 2 264 4856

Mobile telephone number: +32 476 390 822

Fax number: + 32 2 264 4833

E-mail: gilbert.verbeken@mil.be

Abstract Since 1987, the Burn Wound Centre of the Queen Astrid Military Hospital (Brussels, Belgium) has been generating cultures of human epithelial cells (keratinocytes) as an additional surgical tool to treat its critically burnt patients. Initially, the production environment of keratinocyte grafts at the Burn Wound Centre as well as other important aspects that guarantee the safe use of these products on patients (e.g. the donor- and release testing) were regulated solely by national legislation and national quality guidelines. Production units and cell banks were licensed and inspected only by the Belgian national health authorities. In 2004, the European Tissues and Cells Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells [1] was published and later transposed into Belgian Law. For the Burn Wound Centre, implementing this new law meant increased production costs and no significant increase in final quality and/or safety of the produced grafts. In 2007, Europe published Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products [2], amending Directive 2004/23/EC. Overnight, the keratinocytes cultured at the Burn Wound Centre became (arguably) “Advanced” Therapy Medicinal Products (ATMPs) to be produced as medicinal products for human use. The practical impact of this amendment was (and still is) considerable. A similar development appears imminent in bacteriophage therapy. Bacteriophages are bacterial viruses that can be used for tackling the problem of bacterial resistance development to antibiotics. Therapeutic natural bacteriophages have been in clinical use (including in Europe) for almost 100 years. Regulators today are framing the (re-)introduction of (natural) bacteriophage therapy into “modern western” medicine as biological medicinal products, also subject to stringent regulatory medicinal products requirements. In this paper, we look back on a century of bacteriophage therapy to make the case that therapeutic natural bacteriophages should not be classified under the medicinal product regulatory frames as they exist today.

Keywords Bacteriophage; Bacteriophage Therapy; Keratinocytes; Fecal Transplants; Regulatory; Advanced Therapy Medicinal Products; ATMP; Biological Medicinal Products; Hospital Exemption.

1. Introduction

Regulation (EC) No 1394/2007 of the European Parliament and of the Council on Advanced Therapy Medicinal Products (hereafter referred to as "ATMP" Regulation) was adopted in 2007 and covers products that are based on gene therapy, somatic cell therapy or tissue engineering [2]. The ATMP Regulation entered into force in Europe on 30 December 2008. A transitional period was foreseen for ATMPs that were already on the EU market. Gene therapy products and somatic cell therapy products were required to comply with the Regulation by 30 December 2011. Tissue-engineered products were required to comply with the new requirements by 30 December 2012. The new ATMP Regulation gives EU member states the freedom to authorise the production and use of custom-made ATMPs in hospital settings at the member state level as an exemption to the general obligation to follow the central ATMP marketing authorisation procedure. This exemption is called the "hospital exemption". A hospital exemption can be granted for ATMPs that are prepared on a non-routine basis *and* are prescribed for individual patients *and* are applied in a hospital setting *and* on patients that are treated under the professional responsibility of a medical practitioner. Under the hospital exemption, national requirements on quality, traceability and pharmacovigilance *equivalent* to those required for authorised medicinal products are applicable. Before the publication of the ATMP Regulation, dozens of human cell and tissue products were produced and used in European hospitals. In Belgium, for instance, patients had access to 22 cloaked ATMP products produced by 9 accredited and hospital-based (not-for-profit) human cell and tissue establishments [3]. The safe use of these products was guaranteed by national guidelines as well as by Belgium's transposition of the European Cell and Tissue Directive 2004/23/EC [1]. Human-derived cell and tissue products were, at that time, not considered as medicinal products. Under the new ATMP regulatory framework, only 10 marketing authorisation applications for ATMPs have been submitted to the European Medicines Agency (as of 30 June 2013) [4]. Out of these ten marketing authorisation applications, only four have successfully completed the procedure and have been granted a marketing authorisation by the Commission (*ChondroCelect®*, *Glybera®*, *MACI®*, *and Provenge®)*. What happened to the high-quality "hospital-produced" products that

were in use before the publication of the ATMP Regulation? Not-for-profit stakeholders were forced to stop using these products due to the financial strain linked to the production and market placement of medicinal products. Other stakeholders continue to use these ATMPs despite a legal grey zone and an uncertain future.

In Europe, ATMPs have been extensively researched in a clinical context. Up to 250 distinct ATMPs were reported in the European Clinical Trial Database (EudraCT) during the period 2004-2010 [4]. The majority of research on ATMPs is conducted by Small and Medium Enterprises (SMEs) and entities that operate on a not-for-profit legal basis (70%). Big pharmaceutical companies account for less than 2% of all sponsorships [4]. Despite being financially well-equipped and accustomed to investing in pharmaceutical product development, big pharmaceutical companies do not appear to be interested in developing ATMPs.

The Burn Wound Centre of the Queen Astrid Military Hospital (Brussels, Belgium) has been a European pioneer in the defined production of human epithelial skin cultures (keratinocytes) for use on acute and chronic skin wounds for nearly 30 years. Since 1987, the centre has successfully treated more than one thousand patients with keratinocyte-based grafts. As the Belgian definition of an ATMP hospital exemption has not yet been articulated, the centre continues its work within the framework of the European Tissues and Cells Directive 2004/23/EC [1]. Arguably, the hospital should stop producing keratinocytes for clinical use since it does not have a medicinal product production licence, it does not have a pharmaceutical production environment and it does not have a pharmaceutical marketing authorisation license for keratinocytes produced on its premises. Due to this situation, the centre is forced to continue its work in a legally grey zone at the mercy of the National Competent Authority. Important is that the European Commission has now advised the European Parliament to create a more favourable environment for ATMP developers working in an academic or non-for-profit setting [4]. However, it is unlikely that this advice will result in immediate action.

Patients in the Burn Wound Centre of the Queen Astrid Military Hospital (Brussels, Belgium) are not only treated with cultured keratinocytes. They are, since 2007, also sporadically treated with natural

bacteriophages, under the umbrella of Article 37[1] of the Helsinki Declaration [5]. Antimicrobial resistance in bacteria is, also in the Burn Wound Centre, an increasingly serious threat. New initiatives to tackle the problem of antibiotic resistance are urgently needed. One promising solution is the therapeutic use of natural bacteriophages – the viruses of bacteria – to treat bacterial infections. When discovered in the early twentieth century, bacteriophages were immediately applied in medicine (bacteriophage therapy) with variable success. After World War II, Western industry and policymakers preferred antibiotics, which at the time had obvious advantages in terms of breadth of coverage and ease of production and patentability, and bacteriophage therapy was pushed into the background. Today, bacteriophage therapy is again put forward as a potential way to address the current antibiotic crisis. Regulatory parallels can be seen between the regulation history for human keratinocyte-based grafts and that of natural therapeutic bacteriophages. Natural bacteriophages have been used for therapeutic purposes in humans for almost 100 years. Today, Europe classifies therapeutic bacteriophages as human medicinal products to be regulated through the classical human medicinal product frameworks [6]. We urge regulators *not* to repeat the regulatory mistakes of the past. To maximally exploit the advantages bacteriophages have over conventional drugs, it is important that sustainable bacteriophage products are not submitted to the conventional long medicinal product development and licensing pathway. There is a need for an adapted framework, including realistic production and quality and safety requirements, that allows a timely supplying of bacteriophage therapy products for 'personalized therapy' or for public health or medical emergencies [7]. All stakeholders should be aware that they have a moral duty to proceed fast within their respective domains of responsibility in countering bacterial antibiotic resistance [8,9]. We are convinced that opting for a local, patient-specific approach will allow us to strike the right balance between the obligation to protect the patient from unnecessary risks on the one hand and the obligation to offer the best possible medical attention to the patient on the other. Flexibility (at all levels) is a basic requirement for success in this endeavour.

[1] Art. 37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

2. The Belgian Ministry of Defence's historical ATMP experience

The Queen Astrid Military Hospital established a human keratinocyte production unit in the late 1980s. Its principal goal was to produce autologous keratinocyte sheets (see Figure 1) for immediate use on critically burnt patients (mostly civilians). The launch of this production unit (staffed primarily by biologists performing their obligatory military service at that time) was successful and the first patients were grafted in 1987. The technique for growing keratinocytes was referred to as the "Rheinwald and Green" technique [10]. Over time, the technique has been optimised. Keratinocytes can now be grown in totally defined and animal-component-free culture media, without the additional use of animal fibroblasts feeder layers (see Figure 2). This defined production protocol was published open access (without patent protection) in 2012 [11]. Figure 3 illustrates the timeline related to this project (see Figure 3). Alongside culturing autologous cells, donor keratinocytes for allogeneic use are also grown. The cultured keratinocytes can be cryopreserved for later use (see Figure 4). Keratinocytes can be applied under the form of a sheet or under the form of a spray (see Figure 5). It is also possible to generate cultures using adult skin or from neo-natal foreskin donations. The keratinocyte bank of the Queen Astrid Military Hospital became ISO 9001 certified in 2008. The hospital works in compliance with the European Tissues and Cells Directive 2004/23/EC [1] and the keratinocyte bank is compliant with specific Belgian regulation and guidelines as defined by the Belgian Health Authorities and advised by the Belgian Superior Health Council. In addition, the hospital's keratinocyte bank is licenced by the Belgian Federal Public Service for Health, Food Chain Safety and Environment. Initially, the keratinocyte bank was inspected (in view of the prolongation of the licenses) by the Belgian hospital inspection authorities. Inspection duties later transferred to the pharmaceutical inspection authorities, the Belgian Federal Agency for Medicinal and Health Products (FAMHP). The use of keratinocytes for treating burn wounds or chronic skin wounds was (and still is) reimbursed by the Belgian social security system (after having documented the efficacy) at a (not-for-profit) production cost. In 2012, the hospital received a letter from the Belgian National Competent Authority (FAMHP) that these cultured keratinocytes had been reclassified, effective immediately, as medicinal products for human use and were thus to be produced and

placed on the market as if they were human medicinal products. Keratinocytes are cultured currently in a "controlled environment" (GMP air quality Class A in a minimum Class D background). Halting production at the centre meant ceasing all keratinocyte-based treatments, since no equivalent products for keratinocytes are currently available on the market. Faced with this situation, the Belgian Ministry of Defence had no other choice but to invest €5.3 million in a cleanroom facility for GMP (keratinocyte) production. The authors are convinced that this investment only increases production costs and, again, will not increase the quality and safety of the final products.

3. Gleaning lessons on the (re-)introduction of natural bacteriophage therapy in Europe

Parallels can be seen between the regulatory history of keratinocytes and current developments in the re-introduction of (natural) bacteriophage therapy. Europe recently classified natural bacteriophages, used as therapeutics, as human medicinal products [6]. This classification will impact access for patients in a way similar to how the ATMP Regulation impacted patient access to cultured keratinocytes. Therapeutic use of natural bacteriophages (on humans) goes back for almost 100 years [12,13]. Since 2007, the Burn Wound Centre of the Queen Astrid Military Hospital sporadically applies bacteriophage therapy in patients infected with antibiotic-resistant bacteria. The first therapeutic use of bacteriophages in the centre was conducted in a small clinical trial [14] in which bacteriophages were sprayed on the patients' wound bed (see Figure 6). Ten bacteriophage applications in total were performed on 9 patients. This trial was approved by the Leading Medical Ethical Committee of the University Hospital of the Free University of Brussels (UZ Brussel). After the trial, patients in the Burn Wound Centre continued to receive sporadic spray-based and drain-based treatments with natural therapeutic bacteriophages (see Figure 7). These treatments were performed under Article 37 of the 2013 Helsinki Declaration [5]. All patients (or their legal representative) signed an informed consent document. Protocols for the production of natural therapeutic bacteriophages have been increasingly optimised much as keratinocyte production protocols have been optimised over the years. The results of these efforts were published open access (without patent protection) in 2009 [15].

In-house bacteriophage production activities at the hospital are actually not performed in a pharmaceutical GMP environment and the produced natural bacteriophage cocktails are not compliant with the Belgian Medicinal Product Legislation [16]. Bacteriophage cocktail BFC1 (see Figure 8) contains 2 different bacteriophages against *Pseudomonas aeruginosa* (see Figure 9) and 1 phage against *Staphylococcus aureus* .

Within the medicinal product frame, natural bacteriophages are classified as *Biological Medicinal Products* (BMPs). This classification is disadvantageous because the BMPs legal framework does not provide for a hospital exemption procedure, as it does for ATMPs. Big pharmaceutical companies are no longer interested in investing in the development of small-spectrum antibacterial products [17]. The Queen Astrid Military Hospital does not experience the Medicinal Product Legislation as an adequate tool for bringing natural therapeutic bacteriophages to patients in a sustainable and tailored way [9,18,19]. Hospitals working under a - yet-to-be-defined - BMP "hospital exemption" should have access to a specific European Bacteriophage Therapy Legislative Frame that guarantees quality and safety for also these therapeutic bacteriophage products.

4. Fecal microbiota transplantation

In May 2013, the US Food and Drug Administration (FDA) announced that it would begin regulating human feces for transplantation as a "drug" [20,21]. Where the FDA uses the term "drug", Europe uses the equivalent term "medicinal product". The FDA reasoned that this would make fecal microbiota transplantation (FMT) safer by providing oversight, standardising therapy and, eventually, encouraging development of commercial drug products. FMT has been effective in cases of treatment-resistant *Clostridium difficile* infection [22], a killer of 14.000 patients in the U.S. each year.

At a public meeting that month organised by the FDA and the US National Institutes of Health (NIH), patients, physicians and representatives of the Centres for Disease Control and Prevention (CDC) and several professional medical societies voiced concern about restricting access to care. Six weeks later, the

FDA revised its position. The agency decided, for the time being, not to enforce Investigational New Drug (IND) requirements for the treatment of recurrent *Clostridium difficile* infections. This compassionate exception is now enabling many patients to receive much-needed care. Whether and when the therapeutic potential of FMT is realised will depend on how FDA and other agencies regulate the future therapeutic use of stool. Treating stool as a drug imposes strict patient-protection requirements but it significantly limits access to care. Reclassifying stool as a tissue product or giving it its own classification, as the FDA does for blood, would keep patients safe, ensure broad access and facilitate research. We urge European regulators to do the same for natural bacteriophages.

5. Discussion and Conclusion

Today's European Medicinal Product Legislation needs to be reworked to ensure the sustainable larger-scale (re-) introduction of natural bacteriophage therapy in Europe. Defining a hospital exemption under the actual Biological Medicinal Product Legislation is crucial to this effort. This new hospital exemption needs to target the "in-hospital" use of natural bacteriophages, tailored to the patient's needs and taking into account the co-evolutionary aspects of what natural bacteriophage therapy really stands for. Hospitals that have obtained the hospital exemption status should have access to tailored and bacteriophage-specific quality and safety guidelines. Efficacy and safety of the treatments needs to be documented [23] but not necessarily in the format of a clinical trial which exists for the testing and development of new medicinal products. Specific quality and safety requirements should be elaborated to guarantee patients' safe access to efficient bacteriophage therapy products and to facilitate research.

We sincerely hope the arguments put forth in this paper can contribute to effective regulation of bacteriophage therapy within existing regulatory frameworks. Should that fail, a final strategy could be to contend that the tailored use of natural bacteriophages produced in-hospital for use on its own hospitalised patients does not constitute a market placement of that product. Such an interpretation places production of

bacteriophages outside of the scope[2] of European Medicinal Product Directive 2001/83/EC [24] and would render European Medicinal Products Legislation irrelevant to the use of natural bacteriophages inside the hospital on its own patients. A specialised law firm studied this issue in detail and supported this position [25]. Meanwhile, it is possible to revise the actual medicinal product regulatory status of natural therapeutic bacteriophages, allowing compassionate use analogous to the US FMT issue [21].

6. Acknowledgments

The authors want to thank the Leading Medical Ethical Committee of the Free University of Brussels (UZ Brussel) for their open-minded evaluation of the bacteriophage BFC1 clinical trial protocol (2007).

7. Conflict of interest

The authors have no potential conflicts of interest directly relevant to the content of this manuscript.

[2] EMPD 2001; Title II; Scope; Article 2; 1; This Directive shall apply to medicinal products for human use intended to be placed on the market in Member States and either prepared industrially or manufactured by a method involving an industrial process

References

[1] ECTD 2004. European Cell- and Tissue Directive 2004/23/EC of the European Parliament and of the council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells; 2004, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004L0023:EN:HTML .

[2] ATMP 2007. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on Advanced Therapy Medicinal Products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004; 2007, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF .

[3] Pirnay JP, Vanderkelen A, De Vos D, Draye JP, Rose T, Ceulemans C, et al. Business oriented EU human cell and tissue product legislation will adversely impact Member States' health care systems. Cell Tissue Bank 2013;14(4):525-60.

[4] Commission 2014. Commission Report 2014. Report from the Commission to the European Parliament and the Council in accordance with Article 25 of Regulation (EC) No 1394/2007 of the European Parliament and of the Council on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004; 2014, http://ec.europa.eu/transparency/regdoc/rep/1/2014/EN/1-2014-188-EN-F1-1.Pdf .

[5] Helsinki 2013. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. 64th WMA General Assembly, Fortaleza, Brazil, October 2013; 2013, http://www.wma.net/en/30publications/10policies/b3/ .

[6] Parracho HM, Burrowes BH, Enright MC, McConville ML, Harper DR. The role of regulated clinical trials in the development of bacteriophage therapeutics. Journal of Molecular and Genetic Medicine 2012; 6:279-86.

[7] Pirnay JP, Blasdel B, Bretaudeau L, Buckling A, Chanishvili N, Clark J, et al. Quality and safety requirements for sustainable phage therapy products. Pharm Res 2015;PMID: 25585954

[8] Verbeken G, Huys I, Pirnay JP, Jennes S, Chanishvili N, Scheres J, et al. Taking bacteriophage therapy seriously: a moral argument. Biomed Res Int 2014;2014:621316.

[9] Verbeken G, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, et al. Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp (Warsz) 2014;62(2):117-29 .

[10] Atiyeh BS1, Costagliola M. Cultured Epithelial Autograft (CEA) in burn treatment: three decades later. Burns 2007;33(4):405-13.

[11] De Corte P, Verween G, Verbeken G, Rose T, Jennes S, De Coninck A, et al. Feeder layer- and animal product-free culture of neonatal foreskin keratinocytes: Improved performance, usability, quality and safety. Cell Tissue Bank 2012;13(1):175-89.

[12] Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, et al. Phage therapy in clinical practice: treatment of human infections. Pharm Biotechnol 2010;11(1):69-86.

[13] Reardon S. Phage therapy gets revitalized. Nature 2014;510(7503):15-6, Erratum in Nature 2014;510(7504):200.

[14] Rose T, Verbeken G, De Vos D, Merabishvili M, Vaneechoutte M, Lavigne R, et al. Experimental phage therapy of burn wound infection: difficult first steps. Int J Burns Trauma 2014;4(2):66-73.

[15] Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, et al. Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS One 2009;4(3):e4944.

[16] BMPL 2014. March 25th 1964. Belgian Law on Medicinal Products (Compiled; 2014); 2014, http://www.ejustice.just.fgov.be/cgi_loi/change_lg.pl?language=nl&la=N&cn=1964032530&table_name=wet .

[17] Blaser M. Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues. 1st ed. New York: Henry Holt and Company; 2014.

[18] Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, et al. The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm Res 2011;28(4):934-7.

[19] Huys I, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, et al. Paving a regulatory pathway for phage therapy. Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. EMBO Rep 2013;14(11):951-4.

[20] de Vrieze J. The promise of poop. Science 2013;341(6149):954-7.

[21] Smith MB, Kelly C, Alm EJ. Policy: How to regulate fecal transplants. Nature 2014;506(7488):290-301.

[22] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med 2013;368(5):407-15.

[23] Kaźmierczak Z, Górski A, Dąbrowska K. Facing Antibiotic Resistance: Staphylococcus aureus Phages as a Medical Tool. Viruses 2014;6: 2551-70 .

[24] EMPD 2001. European Directive 2001/83/EC of the European parliament and of the council on the Community code relating to medicinal products for human use of 6 November 2001; 2001L0083 - EN - 16.11.2012 - 011.001 - 1 (Compiled; 2014); 2014, http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004481.pdf .

[25] Bredin Prat Lawyers Office. Avis Hôpital Militaire Reine Astrid - Keratinocytes (in French); 2012.

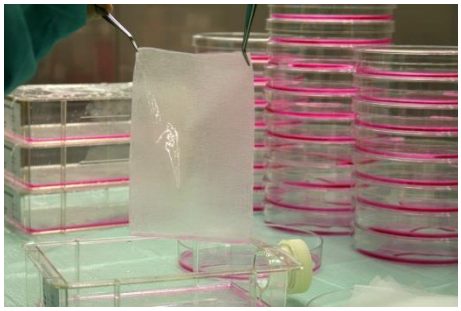


Figure 1 Keratinocyte sheet

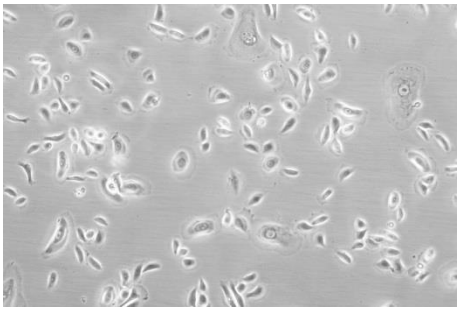


Figure 2 Microscopic view (magnification 40x) of neonatal foreskin keratinocytes

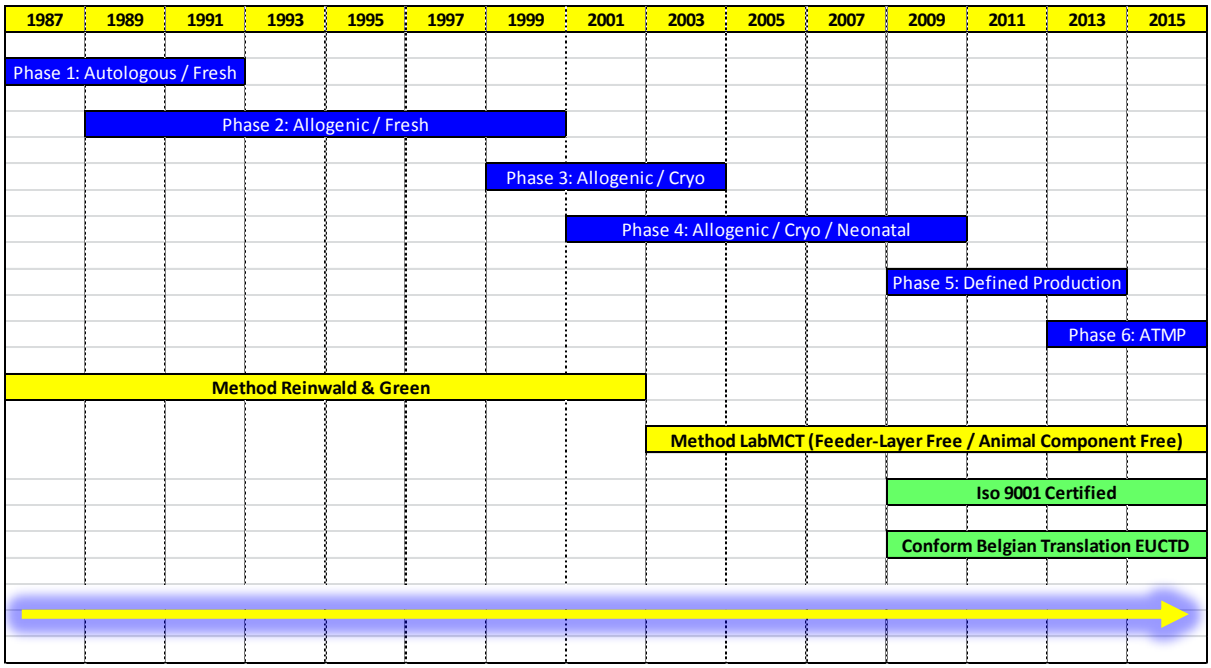


Figure 3 Timeline related to the keratinocyte productions in the Queen Astrid Military Hospital, Brussels, Belgium

Figure 4 Keratinocyte blister package

Figure 5 Keratinocyte spray

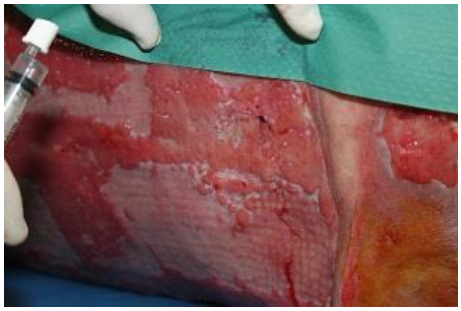


Figure 6 Bacteriophages sprayed on a burn

Figure 7 Bacteriophages applied through a drain in a pelvic traumatic patient under the umbrella of Art. 37 of the Helsinki Declaration

Figure 8 Investigational Bacteriophage Cocktail BFC1

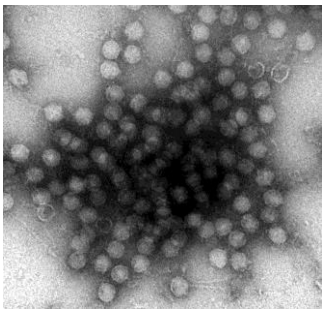


Figure 9 Transmission Electron Microscopy micrograph of *Pseudomonas aeruginosa* bacteriophage PNM

5 Defining a dedicated frame for bacteriophage therapy

5.1 Bacteriophages as medicinal products (Study 8)

5.2 Technical requirements for bacteriophages used as medicinal products (Study 9)

5.3 Validation of the proposed regulatory framework for bacteriophage therapy (Study 10)

5.1 Bacteriophages as medicinal products (Study 8)

5.1.1 Call for a dedicated European legal framework for bacteriophage therapy

G. Verbeken, J.P.Pirnay, R. Lavigne, S. Jennes, D. De Vos, M. Casteels, I. Huys

Arch Immunol Ther Exp. 2014; DOI 10.1007/s00005-014-0269-y

International scientific journal, peer-reviewed

5.1.1 Call for a dedicated European legal framework for bacteriophage therapy

G. Verbeken, J.P.Pirnay, R. Lavigne, S. Jennes, D. De Vos, M. Casteels, I. Huys

Arch Immunol Ther Exp. 2014; DOI 10.1007/s00005-014-0269-y

International scientific journal, peer-reviewed

Arch. Immunol. Ther. Exp.
DOI 10.1007/s00005-014-0269-y

REVIEW

Call for a Dedicated European Legal Framework for Bacteriophage Therapy

Gilbert Verbeken · Jean-Paul Pirnay · Rob Lavigne · Serge Jennes · Daniel De Vos · Minne Casteels · Isabelle Huys

Received: 8 July 2013 / Accepted: 6 November 2013

Abstract The worldwide emergence of antibiotic resistances and the drying up of the antibiotic pipeline have spurred a search for alternative or complementary antibacterial therapies. Bacteriophages are bacterial viruses that have been used for almost a century to combat bacterial infections, particularly in Poland and the former Soviet Union. The antibiotic crisis has triggered a renewed clinical and agricultural interest in bacteriophages. This, combined with new scientific insights, has pushed bacteriophages to the forefront of the search for new approaches to fighting bacterial infections. But before bacteriophage therapy can be introduced into clinical practice in the European Union, several challenges must be overcome. One of these is the conceptualization and classification of bacteriophage therapy itself and the extent to which it constitutes a human medicinal product regulated under the European Human Code for Medicines (Directive 2001/83/EC). Can therapeutic products containing natural bacteriophages be categorized under the current European regulatory framework, or should this framework be adapted? Various actors in the field have discussed the need for an adapted (or entirely new) regulatory framework for the reintroduction of bacteriophage therapy in Europe. This led to the identification of several characteristics specific to natural bacteriophages that should be taken into consideration by regulators when evaluating bacteriophage therapy. One important consideration is whether bacteriophage therapy development occurs on an industrial scale or a hospital-based, patient-specific scale. More suitable regulatory standards may create opportunities to improve insights into this promising therapeutic approach. In light of this, we argue for the creation of a new, dedicated European regulatory framework for bacteriophage therapy.

Keywords Bacteriophage · Therapy · Human · European · Regulatory · Legal · Legislation

G. Verbeken (✉) · J.-P. Pirnay · D. De Vos
Laboratory for Molecular and Cellular Technology, Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium
e-mail: gilbert.verbeken@mil.be

G. Verbeken · M. Casteels · I. Huys
Faculty of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

R. Lavigne
Laboratory of Gene Technology, KU Leuven, Leuven, Belgium

S. Jennes
Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium

I. Huys
Centre for Intellectual Property Rights, KU Leuven, Leuven, Belgium

Introduction

Antimicrobial resistance is a key twenty-first century global health challenge (Cooper and Shlaes 2011; Kutateladze and Adamia 2010). The potential of bacteriophages for treating (multi-drug resistant) bacterial infections has been acknowledged for decennia (Brüssow 2005; Gill and Hyman 2010; Górski et al. 2009a, b; Maura and Debarbieux 2011; Pirnay et al. 2012) and bacteriophage research is being performed intensively worldwide (Ackermann 2012). Bacteriophage therapy was developed mainly in Eastern Europe (Poland) and the former Soviet Republics (Georgia and Russia). A handful of clinical trials have been performed in those countries, as well as in the United States and India (Bruttin and Brüssow 2005; Monk et al. 2010); however, most of

Published online: 06 February 2014

these studies were not carried out according to modern, evidence-based standards of medical research (Parracho et al. 2012). Today, a small number of clinical trials have been carried out and/or are ongoing and bacteriophage therapy is being applied in clinical settings under the purview of specific national regulatory frameworks and/or the Helsinki Declaration (Górski et al. 2009a, b; Kutter et al. 2010).

The lack of a smooth (re-) introduction of bacteriophage therapy in Europe is related to several obstacles within the current European Regulatory Framework (Brüssow 2012; Pirnay et al. 2011; Verbeken et al. 2012; Wright et al. 2009). Meanwhile, the UK's Medicines and Healthcare Products Regulatory Agency has approved a bacteriophage clinical trial (Pirnay et al. 2011), which is now ongoing. In this context, bacteriophages used as therapeutics are considered "biological medicinal products" by European regulators. In the United States, such bacteriophage-based products are handled by the FDA division for vaccines and related product applications (Parracho et al. 2012). This suggests that a non-specific, technical and stringent legislative pharmaceutical framework is likely to be introduced into the field of natural bacteriophage therapy in the near future. Hospitals using bacteriophage-based products to treat hospitalized patients—many of which hospitals have used these products for many years—must now meet the stringent requirements pertaining to "true" human medicinal product development. This is likely to be destructive for the non-profit (tailored) hospital-based use of therapeutic bacteriophages as well as for small and medium enterprises lacking the necessary financial resources to fund the full product development cycle for bacteriophage-based products (Pirnay et al. 2012; Thiel 2004).

Currently, the regulatory aspect of bacteriophage therapy is understudied. No technical, scientific arguments currently exist addressing the question of whether and to what extent bacteriophages fit within the actual definitions and procedures of the existing regulatory framework for human medicinal products in Europe.

This study investigates the scientific arguments related to the classification of bacteriophages as human medicinal products under the current European regulatory framework. The core of the discussions was the European legislation relevant to the therapeutic (*anti-bacterial*) use of natural (*not genetically modified*) bacteriophages in humans.

The aim of the study was to evaluate whether the current European regulatory framework for human medicinal products needs to be adapted with regard to an eventual (re-) introduction of bacteriophage therapy into the European Union.

Methodology

The research focuses on the application of *natural* bacteriophages in a *therapeutic* context. Other possible fields of applications for bacteriophages (e.g., prevention of infections; use as vaccines; use as diagnostic tools; use as a tool to influence cancer or to decontaminate skin grafts) were excluded.

To investigate the extent to which the concept of bacteriophage therapy does or does not fit into the current European regulatory framework for human medicinal products, the existing biomedical-economic literature was reviewed and in-depth interviews with 35 key informants with knowledge and/or regulatory expertise of bacteriophages were carried out. Participants were selected using purposive sampling. The experts represent different stakeholder groups, including industry (11), academia (18), hospitals (5) and competent authorities (1). The interviewees were based in Belgium (15), France (8), United States (3), United Kingdom (2), Georgia (2), Germany (1), Poland (1), Portugal (1), Switzerland (1) and The Netherlands (1).

The interview was based on a standardized questionnaire. Three definitions from the existing European regulatory framework were presented to the interviewees (Boxes 1–3): the general definition of a medicinal product, the definition of a biological medicinal product, and the definition of an Advanced Therapy Medicinal Product (ATMP). The interviewees were asked to comment on how bacteriophages did or did not fit into the wordings of the presented definitions (see Fig. 1). Beside these three main definitions, the following topics where also discussed: the definition of a bacteriophage, whether (or not) a bacteriophage is in fact a living entity, therapeutic quality and safety issues, application methodologies, possible side effects of bacteriophages, differences/similarities of bacteriophage therapy versus antibiotic therapy, marketing authorization pathways, the hospital exemption issue and intellectual property aspects. The interviewees were asked to formulate conclusions about whether (or not) the current European regulatory framework is sufficient, needs to be adapted or whether there is a need for a new, dedicated framework specifically for bacteriophages.

The interviews were qualitatively analyzed and consistent themes and patterns were identified. Due to the complexity of the interview data, results were processed and analyzed using non-computational qualitative methodology (Silverman 2010).

Results

This chapter summarizes the answers/the reflections of the interviewees in relation to the questions asked. These answers, reflections or statements do not necessary reflect the position of the authors of this paper. Literature-references are not included in this chapter since it is not known

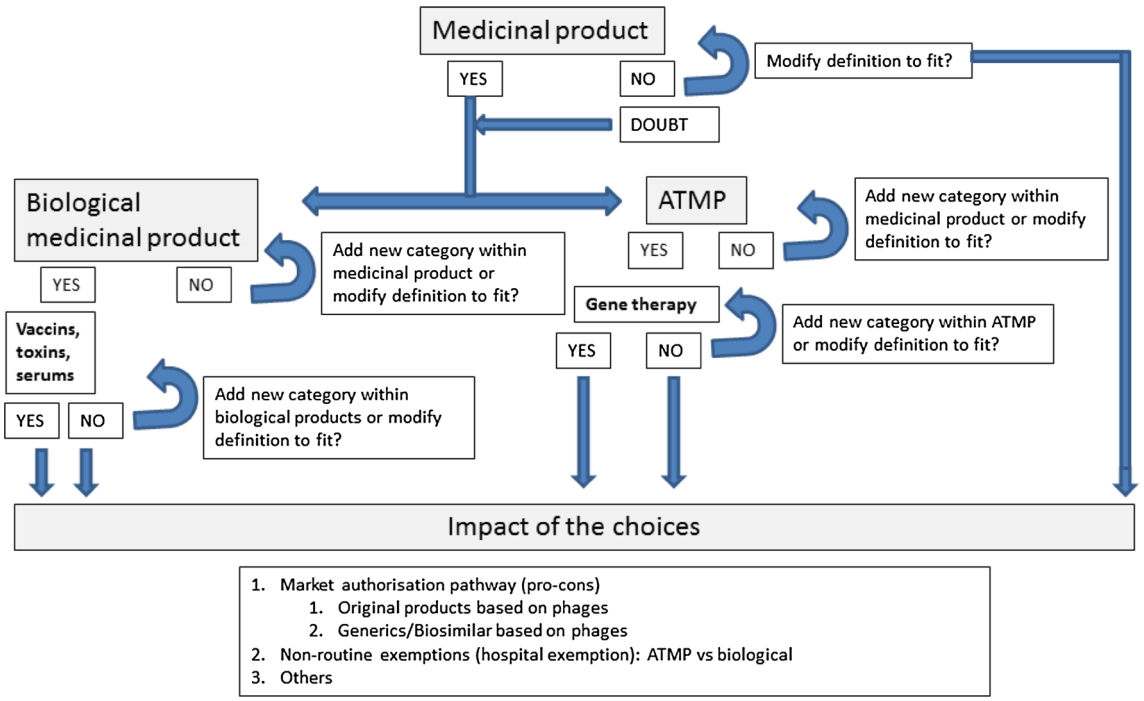


Fig. 1 Overview of methodological approach of the interviews

to the authors from what (publication) background the interviewees were answering the questions.

Bacteriophages as Human Medicinal Products under the European Regulatory Framework

Arguments Related to Bacteriophages and the Definition of a Human Medicinal Product (see Box 1)

The definition of a human medicinal product (Art. 1 of Directive 2001/83/EC) refers to a "substance" (or a combination of substances) presented with particular therapeutic properties and used in therapeutic contexts. Such a substance is also defined in the Directive (Box 1) and is perceived to be "any matter irrespective of origin", with some additional examples. The definition of this referred substance is so broadly defined that it includes natural bacteriophages used as antimicrobial agents within human beings. The definition could even be taken to cover a physician, since a physician is also "presented to patients as having properties for treating disease".

In view of the particular examples of substances (Box 1), opinions differ on what a natural bacteriophage really is. A bacteriophage can be considered a microorganism—or not—and as living—or not. Differences at this conceptual level are important when considering a potential classification of natural bacteriophages in the existing legal framework for human medicinal products.

In the case that a bacteriophage is considered a microorganism, we can refer to it as a bacterial virus, a microbe or some other organism. According to classical taxonomical terminology, a bacteriophage is indeed a virus. A virus outside a bacterium is called a "virion" (an "intermediate phase"). "Virions" can be compared to spores or sperm cells. A spore is not a plant, and a sperm cell is not a human being. Once the virion is inside the bacterium, this bacterium is no longer the same cell. The virion takes over the essential elements and processes within the bacterium. The changed (infected) cell could thus be called a "viral cell". It is the virus-cell combination that then produces the bacteriophages (virions). In this view, the bacteriophage together with its bacterium can be considered to be *one* microorganism, since a bacteriophage has no existence without that bacterium. The *bacteriophage-bacterium combination* could be classified as a new taxonomic entity.

On the other hand, there are arguments supporting the idea that a bacteriophage is not a microorganism since a bacteriophage has no "organs" and requires a cell machinery to be "alive". According to this logic, a bacteriophage can be considered as *derived from* a microorganism.

With respect to its replicating nature, a bacteriophage is perceived as a biological entity that, by interacting with its (biological) environment, is capable of replicating and evolving as an independent, self-replicative particle. But others do not consider a bacteriophage self-replicative

since a bacteriophage needs a host (a biological system) to self-replicate.

Is a Bacteriophage Living? Some do not consider a bacteriophage as a living entity since it lacks the most basic component of a biological system, namely, a cell (the biologic basic entity). A cell is an open thermo-dynamic system with a constant material and energy flow. Being alive implies a status with a "anti-entropic effect". This contrasts with a bacteriophage as a (classically defined) virus consisting only of (static) proteins and nucleic acids. Others consider bacteriophages as living entities. Due to it being a very small entity—a capsuled single-stranded DNA—(the entire DNA of) a bacteriophage was one of the first molecules to be synthesized in the laboratory. Although very simple in design, this piece of DNA is not functional when introduced as such into a bacterium. Therefore, arguments can be found to qualify bacteriophages as "living". Viruses are, after all, part of the "tree of life".

Therapeutic Action of Bacteriophages Bacteriophages can "treat or prevent" a disease in human beings and they "restore, correct or modify physiological functions by exerting pharmacological, immunological or metabolic actions" as described in the Directive (Box 1). Different aspects can be considered with respect to the exact mode of action of a therapeutic bacteriophage. Bacteriophages can *restore* physiological function and the original endemic flora by controlling the pathogens present there. In this way, they can restore balance to out-of-control systems. In cases where different bacteria are involved, bacteriophages can generate a competitive exclusion by specifically attacking a particular bacterium, thus rebalancing the ecosystem.

Another mode of action of bacteriophages relates to their capacity to effectively *modify* human physiological functions, be it in an indirect way, by destroying the bacteria. In this sense, they are comparable with antibiotics. *Immunological* actions can be attributed to bacteriophages by specifically boosting the human immune system. Even a *metabolic action* of bacteriophages can be observed, since bacteriophages interact with the microbial parts of the human body, correcting or modifying physiological functions by killing off pathogenic microorganisms. In addition, bacteriophages take over the bacterial metabolism. Bacteriophages can also generate a *pharmacological* action since bacteriophages are not only antimicrobials but can also suppress inflammation caused by infection.

Finally, bacteriophages can be used as a medical diagnostic tool (Box 1), as was the case when they were used in *salmonella* testing during *salmonella* outbreaks and fast plaque testing for tuberculosis.

Route of Administration of a Bacteriophage-Based Product There is a lack of scientific evidence about the most optimal application format and methodology for bacteriophage therapy. The external (topical) or oral use of bacteriophages should pose no problems. The preferable application method, however, is intra-peritoneal or intramuscular. Bacteriophages are then released into the bloodstream very slowly, gradually and at low levels. In this way, the immune system is stimulated much less than it would be were the bacteriophages to be directly injected intravenously. Once the bacteriophages are at the point of action, they will auto-amplify as needed. Bacteriophages have widely been used intravenously. For instance, the intravenous anti-staphylococcal bacteriophages produced at the Eliava Bacteriophage Institute's industrial department have been used across the whole Soviet Union from the end of 1970s through the end of 1980s for treatment of septic infections in humans (children and adults) caused by multiple drug-resistant *Staphylococcus aureus*. However, there is some sense in not administering bacteriophages intravenously, particularly because bacteriophages are likely to be filtered out by the immune system almost immediately using this method. In an effort to prevent this, one could try to cover the bacteriophages with molecules, making them invisible to the immune system. However, after this manipulation, the bacteriophages can no longer be considered "natural" bacteriophages. Ultimately, while promising as an avenue for further research, bacteriophages may not be suited to treating kidney or liver infections since maintaining adequate bacteriophage concentrations to treat at these locations is probably infeasible.

Possible Side Effects of Bacteriophage Therapy Predicted side effects are very few and mostly depend on the time of administering the bacteriophages, the applied amount of bacteriophages, the type of bacteriophages used, the format of application, and whether the bacteriophages are administered as cocktails.

With respect to genetic (carcinogenic) consequences related to bacteriophage therapy, gene transfer cannot be totally excluded, but will probably only happen at a very low frequency.

Bacteriophages can cross the blood–brain barrier, but no known specific side effects related to this have been reported.

Immunological response at the moment of treatment and immunization against the bacteriophages when used in the long run could also be possible. This phenomenon is not likely to appear when the treatment period is (very) short. This is why repetitive treatment at intervals of several weeks or months (with the same bacteriophages) should be avoided. When using bacteriophage cocktails in a particular therapy, bacteriophages must be changed or updated

frequently and broad-spectrum cocktails must be composed of the least possible number of bacteriophages.

The use of therapeutic bacteriophages will lead to a quick and in some cases quit massive destruction of the bacterial cells involved. At worst, this massive and total lysis of bacteria and the subsequent release of toxins could generate potentially life-threatening reactions such as endotoxin shock, mechanical osmotic effects or respiratory symptoms. The use of small quantities of bacteriophages at once is necessary in order to avoid the large-scale release of toxins. In addition, a first, limited amount of bacteriophages prior to a higher therapeutically relevant dose can prevent large-scale toxin release because the initial bacteriophages destroy the bacteria before they multiply massively.

A side effect of bacteriophage cocktails in particular is the risk of recombination that can occur within a bacteriophage, ending all control over the process. Recombining the genetic information within bacteriophages can modify the original bacteriophages. This happens in nature and is being studied in labs; however, more modeling studies are necessary to fully explore this.

It is clear that the long-term consequences of bacteriophage therapy remain partly unknown, especially in view of the resistance development. Although bacteriophages can adapt and evolve along bacterial changes, research related to the development of resistance in general and research on bacteriophages more specifically is therefore necessary. We must treat carefully and draw on lessons learned in the past from the development cycle of antibiotics, which progressed without any profound, thorough risk assessment. For bacteriophages, the (environmental) risk assessment for (non-human) medical use is also important due to problems that may arise from the massive use of bacteriophages in, e.g., the veterinary, bio-agricultural industry, as was and continues to be the case for antibiotics use in that industry.

Views Related to Bacteriophages and the Definition of a Biological Medicinal Product (see Box 2)

The definition of a Biological Medicinal Product refers to the active substance as a biological substance produced or extracted from a particular biological source. Active products used in natural bacteriophage therapy can be classified under the definition of a Biological Medicinal Product.

This is the case for several reasons. First, a natural bacteriophage itself can be perceived as a *biological substance*. Such bacteriophages can be produced by or extracted from a biological *source*, as proclaimed in the Directive. The bacterium itself can be seen as the biological source. Other possible biological sources are the initial ecological combination 'bacteriophage-bacterial host', or the bacteriophage itself, which enters a bacterial cell, interacts with it and replicates. Even the wound fluid of the patient or the wastewater out of which bacteriophages can be extracted could be viewed as possible biological sources.

It is also possible to view a bacteriophage as not extracted from but *made by* the bacterial cell. The bacteriophage lyses the bacterial cell and releases itself from its host. A bacteriophage has a self-replicating nature, but it can only reproduce (or make) itself when present in a bacterium, namely, a very bacterial-specific host or the biological system to which it belongs.

One could also consider the endozymes produced by the bacteriophage as active biological *substances*. Such endozymes cause lysis of the bacterium and originate from the bacteriophages as a biological *source*.

When bacteriophages are considered as human medicinal products, the *starting material* (as indicated in Directive 2001/83/EC) for producing the therapeutic bacteriophage product must be a substance of biological origin (Box 2). The exact meaning of that starting material can differ. One can consider a microorganism as the starting material for producing a therapeutic bacteriophage, or a particular substance, produced by a microorganism. Another view identifies two types of starting materials for producing therapeutic bacteriophages, namely, "virions" and bacteria, forming bacterio-viruses. Yet another approach is simply to characterize a bacteriophage's parent as its substance of origin.

Physico-Chemical-Biological Testing of Bacteriophages With respect to the characterization and determination of the *quality* of a bacteriophage-based product (Box 2), it could be argued that a combination of physico-chemical-biological testing is required, together with testing of the production process and its control, as described in the Directive (Box 2). However, the exact meaning of physico-chemical-biological testing in view of bacteriophage therapy needs to be clarified, particularly in relation to the required documentation package for bacteriophage therapy.

To generate a qualitative effect of therapeutic bacteriophages, the first requirement is to assess the underlying therapeutic problem of the patient, namely to identify the problematic bacterial strain so that the right corresponding (most effective) therapeutic bacteriophage can be selected.

Once selected, the bacteriophage-bacterium interaction (the *efficacy*) needs to be evaluated in vitro. Electron microscopy can be helpful in documenting the interaction bacteriophage-bacterium.

Bacteriophages need to be characterized in view of the specific *morphotype*. Maximal *molecular characterization* of the bacteriophage genome is mandatory to confirm the

absence of known toxic genes or to confirm the absence of known antibiotic-resistant genes, but not for each batch produced (only for the master stock). In view of the genetic testing of bacteriophages, it could be useful to have a microchip formulation that could be used to test any cocktail to ensure that it is not carrying a pathogenicity island. Full genetic sequencing can, however, lead to false safety statements since, even when a bacteriophage is fully sequenced, half of its genome (and/or related functions) remains unknown. The presence of *lysogenic bacteriophages* must be maximally excluded. In any case, it is also important to point out that a bacteriophage that lacks any lysogenic component can acquire one from a lysogenic bacteriophage that is already present in the body. Bacteriophages arising from host bacteria with the lowest level of emerging *mutations* must be chosen for the production of bacteriophage preparations. When possible, bacteriophages should be produced in a *non-pathogenic bacterial host*, and that host must be sequenced as well. This issue is less (or not) relevant when bacteriophages are grown on the patient's own bacteria. In order to avoid genetic alterations, it would be wise *not* to *scale up* the production of bacteriophages indefinitely. Although bacteriophages are natural products, producing them in high quantities is not natural. Unexpected changes could be introduced. In the case of industrial bacteriophage preparations, permanent monitoring of the production process is seen as mandatory and must be reproducible.

Final bacteriophage preparations must be *pure* (absent of residual contaminating bacteriophages, absent of (other) hosts), *sterile, endotoxin purified* and *pH neutral*. *(Endo) toxin testing* and/or *pyrogenicity testing* of the final products is/are considered necessary. The final *bacteriophage titre* must be tested, as well as the *(storage) stability* (and conditions).

Assessing *pharmaco-kinetics* of the bacteriophage preparations (in relation to the application format, under relevant conditions) is also beneficial. Also the *(adverse) immune response* of the human body should be studied. *In vitro modeling* is important to understanding the action of the bacteriophages.

When bacteriophages are stored in a "therapeutic phage bank", it would be interesting to compare the quality management applied in such master bacteriophage banks with that applied in human cell banks.

In contrast to this rationale, counterarguments state that no elaborated bacteriophage quality and safety documentation is necessary since the safety of bacteriophages has been proven through their long-standing historical use. Bacteriophages are the most abundant form of "life" on earth and are even older than bacteria. If bacteriophages were pathogenic to humans, so goes the argument, it would be publicly known by now. According to this way of thinking, efficacy is all that must be tested and human clinical trials should be conducted. Historical data related to bacteriophage therapy were not, in most cases, generated in accordance with western research standards. Most of these data were collected through "open" clinical trials in eastern countries and lack any written decent reports or data audits. Therefore, in order to be useful, these historical data must be validated. In view of this, it has been suggested to (partially) fall back on these historical data for documenting bacteriophage safety. Efficacy must be proven through standardized clinical trials.

In any case, the documentation of therapeutic bacteriophages is something to take seriously. Data obtained through scientifically sound clinical trials must live up to western standards. It is important to explain (especially to regulators) what is known about bacteriophages and their therapeutic use and to define acceptable risks of bacteriophage therapy.

In the future, basic sequencing research should be performed to see whether lytic bacteriophages could ever turn into a lysogenic state. In addition, the question of how bacteriophages can adapt to existing natural beneficial bacteria—and what the consequences of such an adaptation could be—should also be addressed. It remains uncertain whether and how bacteriophages can infect eukaryotic cells. Basic studies in vitro have to be validated in vivo. The performance of bacteriophages in vivo can vary from their in vitro activity. In addition, blood–brain barrier crossings must be studied in humans as well as in mice. Evolutionary models have also proven to be important to the study of specific interactions between bacteria and bacteriophages.

Natural Bacteriophages Comparable to Vaccines or Toxins? From a regulatory point of view, bacteriophages are most similar to a particular type of biological medicinal products, namely *vaccines*. More in particular, bacteriophage cocktails used in humans need to be updated over time, especially when bacterial resistance develops (as is the case with the flu vaccine). For that reason, some bacteriophage companies are now liaising with the vaccine unit of the European Medicines Agency (EMA).

However, bacteriophages are not "regular" *vaccines* that are mostly used preventive to produce active immunity and therapeutic only in particular cases. Bacteriophages on the contrary are antimicrobials, with a secondary competence of boosting the immune system, be it in a non-specific manner. Since bacteriophages (or their lysates) can boost the immune system (in different ways), they can effectively be seen as "therapeutic vaccines". Using bacteriophages in this way implies the concept of "auto-vaccination" via bacteriophages, meaning vaccinating patients with their own bacteriophages. Parallels exist in this sense between

bacteriophage therapy and tumor vaccination. In the latter, the patients' own tumor tissue is taken for preparation of the vaccine and the patient's own immune response towards its own tumor is modified. This immune response should be self-limiting since the reaction has to stop when the tumor is gone.

Triggering the immune system is not necessarily positive for the bacteriophage itself since it can be eliminated by the immune system of the patient before destroying the bacteria. On the other hand, bacteriophages can be used to test the state of immunity of the patient in general.

With respect to the category of *toxins*, bacteriophages are not toxic and hence they are not to be considered *toxins* as described in the Directive 2001/83/EC.

Views on Bacteriophages and the Definition of an ATMP (Box 3)

ATMPs are defined in Directive 2001/83/EC as complex therapeutic products. Natural bacteriophages can be considered as *complex products*. Once administered to the patient, control over the stability of the bacteriophage products is lost. When bacteriophages are applied in the wound-bed of the patient, bacteriophages can replicate in bacteria and bacteriophage-variations and mutations can develop. Pharmacokinetics of administered bacteriophages (absorption process) is very complex. Sequencing and determining the exact function (e.g., proteomics) is also complex, as is determining the way bacteriophages realize their therapeutic effect.

However, the actual categories within the ATMP framework (*products for gene therapy, somatic cell therapy or tissue engineering*) are not suitable to natural therapeutic bacteriophages. For instance, natural bacteriophages are not gene therapy medicinal products since they are not genetically modified. For obvious reasons, bacteriophages are not considered somatic cells therapy medicinal products nor tissue engineered medicinal products.

For most complex therapeutic products, a precise legal definition is required. Since natural bacteriophages are already present in nature and in our body, it is questionable whether such a definition is necessary for bacteriophages. The complexity is of a technically different nature than gene or somatic cell therapy. Bacteriophages could be compared to more widely used strategies for improving microbial ecology such as probiotics.

Differences and/or Similarities of Bacteriophage Therapy Versus Antibiotic Therapy.

At the product level, antibiotics are (mostly) synthetically prepared chemical products, although antibiotic compounds isolated from nature exist as well. Natural bacteriophages are (by definition) natural "products".

In terms of function, both antibiotics and bacteriophages modify (indirectly) human physiological functions by destroying pathogenic bacteria. Some antibiotics act at the genetic level while others block specific metabolic pathways. Therapeutically relevant bacteriophages, which are lytic natural bacteriophages, kill bacteria by other mechanisms. Such bacteriophages destroy the bacterium "from the outside" by massively perforating the cell membrane, or "from within" by multiplying within the bacterium and eventually being released from that bacterium. Some modern antibiotics cause lyses of the bacteria as well. Endotoxins are released within the patient through lyses or bacterial cell death in general. In the case of antibiotics, this release almost never causes a major problem for a patient confronted with major (resistant) infections, which is what can be expected for bacteriophage therapy as well.

An important difference between bacteriophages and antibiotics is that bacteriophages have a much more specific, targeted action. The broadest-spectrum bacteriophage will never execute as wide an action as the most targeted antibiotic product does. Therefore, bacteriophages do not disturb the natural flora as much as antibiotics do. However, bacteriophages' high specificity can also be considered a negative factor for clinical application. Another important difference is that bacteriophages are able to diffuse in small numbers to the site of bacterial infection and then multiply only when needed. Antibiotics, on the other hand, must be administered in high doses right at the site of treatment, which may cause collateral damage to the patient.

In contrast to antibiotics, bacteriophages can cross the blood–brain barrier (in small quantities) and perform their action (massively) once the target bacteria are reached. Another advantage of bacteriophages over antibiotics is the reduced risk for development of resistance. The amount of bacteriophages does not decrease when approaching the bacterial target. Distinct from antibiotics, bacteriophages have an additional capacity to act on biofilms since their lysins can destroy the biofilm.

In view of those differences and similarities, most experts agree that bacteriophages and antibiotics should be used complementarily/synergistically.

Views with Respect to (Marketing) Authorization for Bacteriophage Therapy

Two Regulatory Pathways According to the interviewees, two (complementary) regulatory pathways should be defined for bacteriophage therapy.

The first is a regulatory path for a uniform product market placement of natural bacteriophage-based products. Since bacteriophages are regarded as human medicinal

products, the actual legal framework for human medicinal products in Europe is applicable, implying submitting a full product dossier (complying with Directive 2001/83/EG) and conducting large-scale expensive clinical trials. This path imposes several hurdles: (1) The high financial threshold cannot easily be overcome by public stakeholders such as hospitals without financial support from other (government) sources. (2) The current Directive 2001/83/EG provides insufficient technical guidance and legal certainty for the development of products for natural bacteriophage therapy. (3) The primary aim of public stakeholders is in fact not a real "market placement" or "marketing authorization" of a bacteriophage-based (or any other) product. (4) One major risk of such market placement of bacteriophage based products is the development of large-scale resistance, at least when use is widespread and uncontrolled. One suggestion could be to update a standard bacteriophage cocktail preparation on a yearly basis once it is on the market and resistance begins to develop, as is done with the flu vaccine.

The second regulatory path should imply an approach applicable to tailored, patient-specific treatments.

The question arises as to whether hospitals applying a tailored bacteriophage therapy approach in close collaboration with microbiological labs should be excluded from the conventional marketing authorization requirement as described in Directive 2001/83/EG and national laws. Certain non-profit-driven hospitals often possess clinical expertise to provide bacteriophage therapy but lack the financial capacity and interest to engage themselves in large-scale market placements of authorized bacteriophage products. In addition, the bacteriophage itself is not a "product to be brought to the market" (citing the wordings of Directive 2001/83/EC) and the tailored hospital-based bacteriophage therapy approach is the only approach that in reality fully exploits the clinical potential of a therapeutic bacteriophage. Bacteriophage therapy is in fact a therapeutic concept.

If patient-specific use of bacteriophage therapy in hospitals is made exempt from the regulatory framework designed to receive marketing authorization (similar to the hospital exemption rule within the regulatory framework of advanced cell and tissues, ATMPs), quality and patient safety must be guaranteed. It is also argued that not only hospitals but also industry, with specific approval from regulators, should in theory be able to deliver "out-of-frame" and "tailored" bacteriophage preparations to patients and hospitals on a per-request basis. However, the difficulty and expense of applying the "one product for one patient" model is not cost-efficient.

In view of this, it may be more prudent to regulate bacteriophage therapy via a simplified marketing authorization framework, feasible for hospitals as well, by strictly defining the (often rare) indications for bacteriophages uses. For industries interesting in market approval for bacteriophage cocktails, endeavors to work under the orphan drug legal frameworks should be explored.

Over-the-Counter Distribution In view of distribution, there are arguments for a very "liberal" distribution model for natural bacteriophages intended for therapeutic use. Some argue that "over-the-counter" distribution of bacteriophages will increase resistance development, while others argue that "over-the-counter" distribution should be possible on the grounds that solely hospital-based use cannot preclude the development of resistance. Next, others claim that limiting distribution to those who are tested is unrealistic, since testing all patients before allowing them to take bacteriophages would be expensive and economically infeasible. Such pre-testing is not readily available for other conventional drug therapies either.

A consensus solution could be to organize over-the-counter distribution of standard bacteriophage-based cocktail products specifically selected for non-life-threatening infections while leaving treatment in life-threatening situations to tailored bacteriophage-based products in a hospital environment. Most ideal would be hospital-based (lab-linked) and accessible (cheap) use of natural bacteriophage-based products. National "bacteriophage therapy centers" (scientific boards included) as are now being set up in Brussels, could be of great value, in preference when linked to a "therapeutic bacteriophage bank" (e.g., DSMZ—http://www.dsmz.de) where specific bacteriophages could be stored and produced as needed. For any treatment, patients must be tested for the best strain match. Individualized approaches and flexibility for physicians to treat patients via personalized schemes should be central.

Views on an Adapted or New Legal Framework for Bacteriophage Therapy

Stakeholders are convinced of a need for a dedicated (new) regulatory framework for bacteriophage therapy that acknowledges the specific properties of bacteriophages and their bacterial interaction as well as the role of hospitals as providers of bacteriophage therapy. As explained above, bacteriophages are uniquely different from conventional human medicinal products (such as chemical substances, somatic cell therapy products and gene therapy medicinal products, among others) currently regulated under existing frameworks. In view of the fact that even products for homeopathy have a dedicated legal framework, some question why bacteriophage therapy is not regulated in a specified, dedicated way.

An adapted regulatory framework could, for instance, be inspired by the existing legislation governing "advanced cellular and genetic therapy" (ATMPs), where regulators took a binary approach towards industrial as well as hospital-based use of cell and gene products and therapies.

Next to the two-way regulatory paths, a new or adapted framework for bacteriophage therapy must in addition take into account the different trajectories for storing and making available therapeutic bacteriophages. (1) A first possibility would be to use the patient's own bacteriophages in a tailored approach for that individual patient. No long-term storage of bacteriophages would be necessary, but on-site testing facilities would have to be present wherever this kind of tailored therapy is offered ("bacteriophage therapy centers"). (2) A second approach comprises the isolation and storage of well-defined (GMP-produced) therapeutic bacteriophages in a bacteriophage bank, which would then be distributed as needed. Such bacteriophages can represent starting materials for the preparation of a cocktail that could be used for combating broad-scale bacterial infections, e.g., in refugee camps confronted with dysentery. At best, different bacteriophages targeting the same bacterium would be collected and, if necessary, provided for therapeutic use, minimizing resistance issues. (3) Such therapeutic bacteriophages stored in a bank could be ordered as well by a physician for tailored-use within a hospital.

An adapted or new regulatory framework for bacteriophage therapy must guarantee safety and quality. Regulatory conditions that govern the production of human medicinal products (e.g., Good Manufacturing Practices) impose high costs and are perhaps not necessary to increase the safety of bacteriophage-based products. Instead, specific guidelines solely directed at quality and safety of bacteriophage preparations should be developed, harmonized and controlled.

If regulators and legislators are to adapt existing legislation (and its interpretation), public as well as private stakeholders must agree on what type of pathways and approaches need to be developed. All partners in these discussions will eventually come to a consensus understanding on the use of therapeutic bacteriophages and that this understanding will serve as a basis for moving forward in a constructive way.

While regulatory frameworks are (and should be) the product of negotiations with regulators and legislators, the negotiation process takes time; time that is precious given the acuteness of the problems faced. In view of the fact that EMA recognized the regulatory framework of biological medicinal products as applicable to bacteriophages, this regulatory pathway might just be the best place to start for further elaboration. Since the regulatory frameworks relevant to the development of bacteriophage therapy are actually more reasonable in, e.g., Australia, Canada, it is time for Europe and individual European countries to take action. At the same time, an international platform should ensure that international harmonization develops.

Patenting Bacteriophage-Related Applications

Isolated, therapeutic bacteriophages can in theory be patented when a complete, well-defined documentation package is available for the specific bacteriophage(s). This package comprises data related to the genome sequence, pre-clinical information, specific functionality, and specific application, among other features. Inventive steps can be defined on the basis of molecular characteristics, application methodology and eventually production procedures. In practice, patents on bacteriophage products are important tools for attracting investors to new companies keen on developing therapeutic bacteriophages.

Similar to most vaccine patents, a patent for a regularly updated bacteriophage cocktail can also be sought.

Companies interested in placing therapeutic bacteriophages on the market take care of IP: they first choose their most appropriate market niche, gain experience from a regulatory point of view and acquire a first return on investment. In a next step, after building more experience on the subject, expansion to other markets can proceed. IP protection is important in order to be able to develop this pathway.

Discussion

Directive 2001/83/EC defines a human medicinal product, the types of action, its sources and its starting materials. This definition is formulated rather broadly, encompassing natural bacteriophages. For instance, for the products covered by its scope, the Directive does not differentiate between "*direct*" or "*indirect*" therapeutic actions. Bacteriophages generate their action on the patient in an indirect way, similar to antibiotics. Bacteriophages destroy the bacterial pathogen and consequently eradicate or decrease the pathogenic bacterial load in the patient (Payne and Jansen 2003).

It is clear from our analysis that natural bacteriophages fit into the definition of a "biological medicinal product" (Box 2). However, different *biological sources* for the production of a therapeutically active bacteriophage are possible. Since the definition of a biological medicinal product does not limit the types of potential biological sources, therapeutic bacteriophages also comply with the definition of a biological medicinal product. However, therapeutic bacteriophages do not fit into the Special Frames (indicated in the Directive 2001/83/EG) applicable

to biological medicinal products, such as vaccines, toxins and serum-derived products.

In view of the applicability of the ATMP definition (in Regulation 1394/2007) to bacteriophages, it is not clear whether bacteriophages are "complex therapeutic products with technical specificities requiring precise legal definitions" (as is true for ATMPs). In a sense, the regulatory pathways developed for "natural" ATMPs might provide a historical reference point. Products used in somatic cell therapy, when substantially manipulated or used in a non-homologous way, are classified as ATMPs. The human medicinal product Directive 2001/83/EC defines "cultivation", for instance, as a substantial manipulation. Consequently, natural bacteriophages, when cultivated, could also be seen as fitting within the ATMP framework since they would, according to this definition, be substantially manipulated.

Impact of Classifying Natural Bacteriophages as Human Medicinal Products

The development of a human medicinal product, either as a biological medicinal product (Dir 2001/83/EG) or as an ATMP (Dir 2001/83/EG and Regulation 1394/2007) requires huge investments of time and money. The non-profit sector and the diverse interested small and medium enterprises can hardly afford this pathway without external investments. Therefore, there is a need for products like natural bacteriophages to be exempted from the scope of the regulatory framework applicable to human medicinal products, more specific Directive 2001/83/EG and Regulation 1394/2007, depending on whether bacteriophages are seen as biologics or ATMPs.

One way would be not to formulate the therapeutic action of the bacteriophage as a primary mode of action, arguing that such a product is not a human medicinal product. However, this is not the most optimal scenario when the ultimate goal of the exercise is "to bring therapeutic bacteriophages to the patient" (Międzybrodzki et al. 2012; Soothill 2013; Wittebole et al. 2013). In addition, by reviewing the definitions, all reviewers acknowledged that a bacteriophage may fit into the definition of a human medicinal product.

Another way is to use exemptions within the existing regulatory framework for human medicinal products. If natural bacteriophages are considered ATMPs, the ATMP Regulation 1394/2007 is applicable. This framework only specifies certain categories, human somatic cell therapy, gene therapy and tissue engineering. A specific category "viral therapy" could theoretically be introduced under this ATMP framework. In any case, the ATMP Regulation 1394/2007 provides a possibility for hospitals to be exempted from a stringent centralized marketing authorization, referred to as the "hospital exemption" (Art. 28 of Regulation (EC) No. 1394/2007). National rules apply to hospital exempted-ATMPs. However, if present, such rules are in any case specifically designed for cell and tissue based therapies, not bacteriophage therapy. In addition, often such national rules require similar GMP as requested for fully centralized marketing authorization dossiers. Therefore, a specific Directive covering natural bacteriophage therapy (to be implemented in national laws specific for bacteriophage therapy) is desirable.

If natural bacteriophages are considered biological human medicinal products, Directive 2001/83/EG applies. Unfortunately, the Directive 2001/83/EG (currently) does not provide for a hospital exemption (as in the ATMP Regulation). But Article 3 (Paragraph 1) of Title II of the Directive states that it "*shall not* apply to any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient (commonly known as the magisterial formula)." However, a (hospital) pharmacist is not supposed to use non- (EU) licensed products as components for magisterial preparations. Since natural bacteriophages are not licensed products at the moment, this potential pathway could be difficult to implement.

Another way to escape the marketing authorization requirement is to consider the scope of that Directive. Article 2, Par. 1 (Title II) of the Human Medicinal Product Directive states that it "shall apply to medicinal products for human use *intended to be placed on the market* in Member States and either *prepared industrially* or manufactured by a method *involving an industrial process*". As highlighted by the interviewees, a tailored (natural) bacteriophage production (Merabishvili et al. 2009), performed within a hospital for use on particularly defined patients can hardly be seen as an "industrial production". In addition, the therapeutic, in-house use of these produced bacteriophages is not "market placement". Hospitals are not interested in producing human medicinal products for the purpose of obtaining a "marketing authorization" for further distribution. For these reasons, and analogous to the logic developed in the field of the cellular ATMPs, the tailored production and therapeutic use of natural bacteriophages on humans would appear *not* to be covered by the scope of the Human Medicinal Product Directive. The industrial productions of uniform bacteriophage products intended for European market placement, on the other hand, *are* covered by the scope of this Directive.

If natural bacteriophages would be covered by the scope of the Directive 2001/83/EG, a new hospital exemption needs to be designed for biological human medicinal products, accompanied by a specific Directive for bacteriophage therapy (to be implemented in national laws).

It is clear from the above that there is a regulatory gap for natural bacteriophage therapy. While regulators are

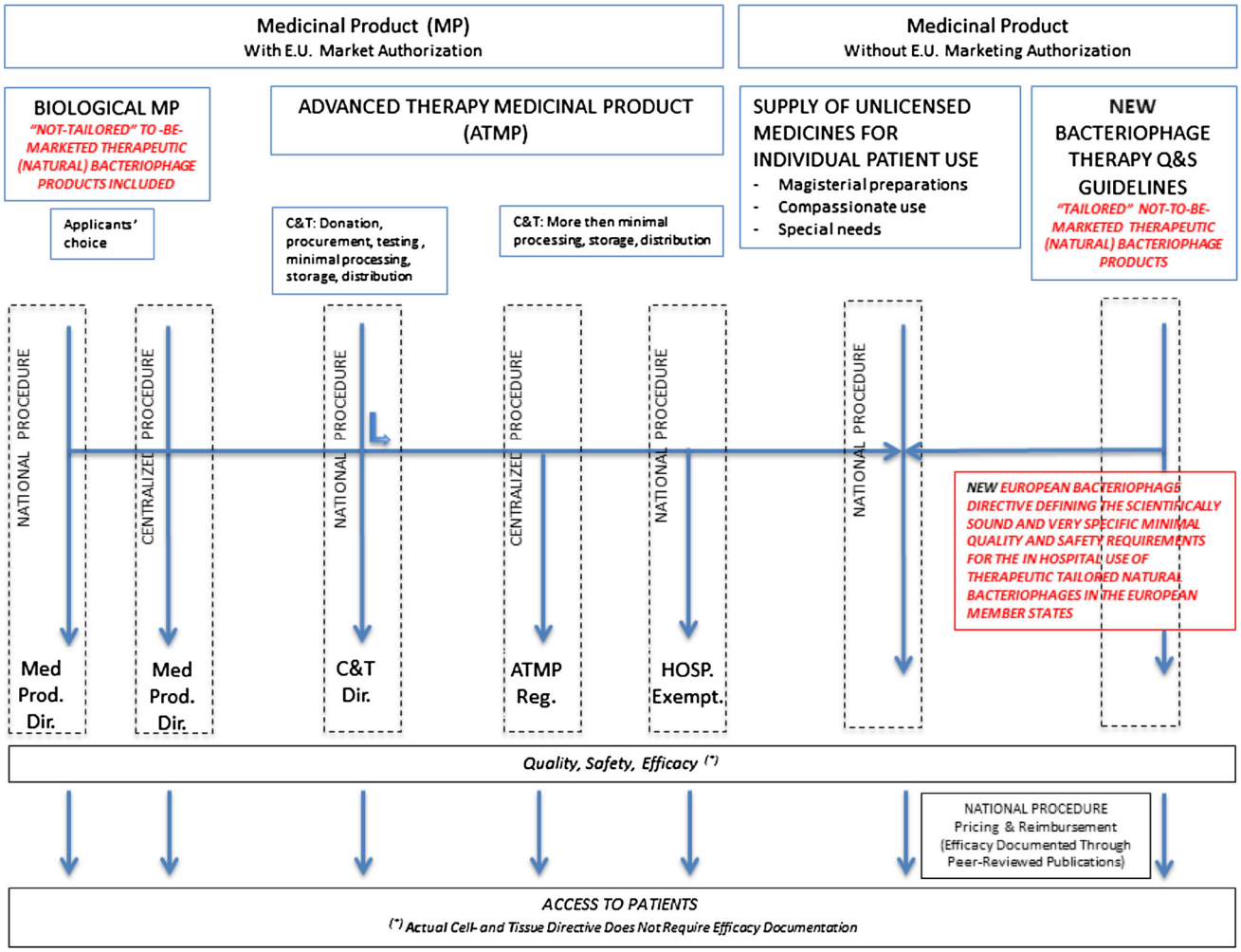


Fig. 2 Proposal for a new European directive for bacteriophage therapy

responsible for applying a regulation, the regulation itself can only be changed through legislative action, which is in this case highly needed to guarantee a timely, flexible and sustainable way of introducing bacteriophage therapy in Europe.

The appropriate legal action we suggest is a European wide Directive for Natural Bacteriophage Therapy. Such Directive should regulate documentation requirements of safety, potency, purity and toxicity, specific in the context of hospital based patient-tailored (natural) bacteriophage therapy. (Industrial) stakeholders aiming to bring pharmaceutical products based on natural bacteriophages to the market could be exempted from the scope of the new Directive and follow the classical medicinal product approach instead (see Fig. 2).

The creation of a bacteriophage-specific Directive could find inspiration on the evolution of what has transpired the last several decennia in the field of human cell and tissue engineered products. As early as the seventies, hospitals were using processed human cells and tissue in treatments for their patients, in accordance with the respective national legislation, until the European Human Cell and Tissue Directive was published in 2004 (Directive 2004/23/EC of the European Parliament and the Council of 31 March 2004). This Directive still applies to all work with human body material today and focuses mainly on the hospital-based development and use of cellular products. A few years later, in 2007, the ATMP Regulation (EC) No. 1394/2007 came into force. This ATMP regulation focused (and still does) mainly on industrial work with human bodily material. At the same time, it allows for a "hospital exemption". The hospital exemption applies to non-industrial, tailored and hospital-based clinical use of cell and gene based ATMPs. Industrial ATMPs meant for market placement are regulated at the European level while hospital-based (non-industrial) productions are regulated at the national level.

In a similar way, the bacteriophage-specific regulatory framework with its specific Directive should (1) distinguish between hospital-based (tailor-made) use of natural therapeutic bacteriophages in patients on the one hand and

industrial production and distribution of uniform bacteriophage products on the other, (2) define specific quality and safety criteria relevant to the use of natural bacteriophages on patients (Merabishvili et al. 2009; Wright et al. 2009), (3) define a specific efficacy documentation package relevant to (natural) bacteriophages, (4) make it possible to give patients in need instant access to natural bacteriophage therapy (Caplin 2009), (5) only define requirements relevant to natural bacteriophages, and (6) fully exploit the co-evolutionary aspects of natural bacteriophages (Krylov 2011; Levin and Bull 2004; Scanlan and Buckling 2012).

Close dialogue, open discussions and information exchange with the EMA and national authorities is crucial. It is thus of high importance that regulators and legislators (Members of Parliament) be persuaded of the prudence of a dedicated Bacteriophage Therapy legal framework for Europe.

Conclusions

A dedicated European Bacteriophage Therapy Legal Framework is a prerequisite for paving the way to the smooth introduction of natural bacteriophage therapy into western medicine. If Europe refuses to support the short-term (safe) implementation of "hands-on" bacteriophage therapy in its member states, the national authorities of the member states should step into assert their responsibility in this respect. Antibiotic resistance is an acute problem, both in public health terms and socio-ethical terms. 25 000 Europeans die each year as a direct consequence of untreatable bacterial infections (Ackermann 2012). There is an urgent need for national bacteriophage therapy centers. Industry can play an important role in this. When bacteriophage therapy centers are unable to (financially) launch themselves, national governments should provide sufficient support and/or stimulate the creation of new pharmaco-economic environments.

Box 1. **Definition of a Human Medicinal Product within the Directive 2001/83/EC of the European Parliament and the Council of 6 November 2001 on the Community code relating to medicinal products for humans use. Consolidated | 2001L0083-EN-21.07.2011-010.002-1. Words written in *Italic* are subject to interpretation, as discussed in the manuscript.**

According Art. 1(2) of the Medicinal Product Directive 2001/83/EC, a Human Medicinal Product is a *substance* or a *combination of substances* presented as having properties for *treating* or *preventing* disease in human beings. According the same Directive, a Medicinal Product *can also be* a *substance* or a *combination of substances* which may be used in or *administered* to human beings either with a view to *restoring*, *correcting* or *modifying* physiological functions by exerting a *pharmacological*, *immunological* or *metabolic* action, or to making a *medical diagnosis*. The *substance* referred to in the Directive is *any matter* irrespective of origin which may be: human, e.g., human blood and human blood products; animal, e.g., *microorganisms*, whole animals, parts of organs, animal secretions, toxins, extracts, blood products; vegetable, e.g., microorganisms, plants, parts of plants, vegetable secretions, extracts; chemical, e.g., elements, naturally occurring chemical materials and chemical products obtained by chemical change or synthesis.

Box 2. **Definition of a Biological Medicinal Product. Words written in *Italic* are subject to interpretation, as discussed in the manuscript**

According to Part I Module 3 (3.2.1.1) of the Medicinal Product Directive 2001/83/EC, a Biological Medicinal Product is a Medicinal Product of which the active substance is a *biological substance*. A biological substance is a substance that is *produced by* or *extracted from* a *biological source* and that requires for its *characterization* and the *quality determination* a combination of physico-chemical-biological testing, together with the production process and its control. The Directive provides specific requirements for particular biological medicinal products such as *vaccines*, *toxins* and *sera*, in particular, for agents used to produce active immunity, such as cholera vaccine, BCG, polio vaccines, smallpox vaccine; agents used to diagnose the state of immunity, including in particular tuberculin and tuberculin PPD, toxins for the Schick and Dick Tests, brucellin; and agents used to produce passive immunity, such as diphtheria antitoxin, anti-smallpox globulin, antilymphocytic globulin. According the Directive (Part I Module 3 (3.2.1.1), the *starting materials* of a Biological Medicinal Product shall mean *any* substance of biological origin such as microorganisms, organs and tissues of either plant or animal origin, cells or fluids (including blood or plasma) of human or animal origin, and biotechnological cell constructs (cell substrates, whether they are recombinant or not, including primary cells).

Box 3. **Definition of Advanced Therapy Medicinal Product (ATMP) from Regulation (EC) No 1394/2007 of the European Parliament and the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 | Official Journal of the European Union | 10.12.2007 | L324:121-137. Words written in *Italic* are subject to interpretation, as discussed in the manuscript.**

The Regulation 1394/2007 on ATMPs (Recital 3) describes ATMPs as *complex therapeutic products* with *technical specificities* requiring *precise legal definitions*. Under the ATMP Regulation, Products for Somatic Cell Therapy, Tissue Engineered Products as well as Gene Therapy Medicinal Products (GTMPs) are classified as ATMPs. According to Part IV of Annex I to Directive 2001/83/EC, a Gene Therapy Medicinal Product is a biological medicinal product that has the following characteristics: (a) It contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; (b) Its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence. Gene therapy medicinal products shall not include vaccines against infectious diseases.

Acknowledgments The authors want to thank all interviewees for their honest, thoughtful and constructive cooperation.

Conflict of interest The authors have no potential conflicts of interest directly relevant to the content of this manuscript.

References

Ackermann HW (2012) Who went into phage research? Bacteriophage 2:55–59

Brüssow H (2005) Phage therapy: the *Escherichia coli* experience. Microbiology 151(Pt 7):2133–2140

Brüssow H (2012) What is needed for phage therapy to become a reality in Western medicine? Virology 434:138–142

Bruttin A, Brüssow H (2005) Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob Agents Chemother 49:2874–2878

Caplin J (2009) Bacteriophage: old treatment, new focus? Microbiologist 10:20–23

Cooper MA, Shlaes D (2011) Fix the antibiotic pipeline. Nature 472:32

Gill JJ, Hyman P (2010) Phage choice, isolation, and preparation for phage therapy. Curr Pharm Biotechnol 11:2–14

Górski A, Międzybrodzki R, Borysowski J et al (2009a) Bacteriophage therapy for the treatment of infections. Curr Opin Investig Drugs 10:766–774

Górski A, Targońska M, Borysowski J et al (2009b) The potential of phage therapy in bacterial infections of the eye. Ophthalmologica 223:162–165

Krylov V (2011) Use of live phages for therapy in a background of co-evolution of bacteria and phages. Int Res J Microbiol 2:315–332

Kutateladze M, Adamia R (2010) Bacteriophages as potential new therapeutics to replace or supplement antibiotics. Trends Biotechnol 28:591–595

Kutter E, De Vos D, Gvasalia G et al (2010) Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 11:69–86

Levin BR, Bull JJ (2004) Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol 2:166–173

Maura D, Debarbieux L (2011) Bacteriophages as twenty-first century antibacterial tools for food and medicine. Appl Microbiol Biotechnol 90:851–859

Merabishvili M, Pirnay JP, Verbeken G et al (2009) Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS ONE 4:e4944

Międzybrodzki R, Borysowski J, Weber-Dabrowska B et al (2012) Clinical aspects of phage therapy. Adv Virus Res 83:73–121

Monk AB, Rees CD, Barrow P et al (2010) Bacteriophage applications: where are we now? Lett Appl Microbiol 51:363–369

Parracho HM, Burrowes BH, Enright MC et al (2012) The role of regulated clinical trials in the development of bacteriophage therapeutics. J Mol Gen Med 6:279–286

Payne RJ, Jansen VA (2003) Pharmacokinetic principles of bacteriophage therapy. Clin Pharmacokinet 42:315–325

Pirnay JP, De Vos D, Verbeken G et al (2011) The phage therapy paradigm: Prêt-à-porter of sur-mesure? Pharm Res 28:934–937

Pirnay JP, Verbeken G, Rose T et al (2012) Introducing yesterday's phage therapy in today's medicine. Future Virol 7:379–390

Scanlan PD, Buckling A (2012) Co-evolution with lytic phage selects for the mucoid phenotype of *Pseudomonas fluorescens* SBW25. ISME J 6:1148–1158

Silverman D (2010) Doing qualitative research. Saga, London

Soothill J (2013) Use of bacteriophages in the treatment of *Pseudomonas aeruginosa* infections. Expert Rev Anti Infect Ther 11:909–1015

Thiel K (2004) Old dogma, new tricks—21st century phage therapy. Nat Biotechnol 22:31–36

Verbeken G, Pirnay JP, De Vos D et al (2012) Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine. Arch Immunol Ther Exp 60:161–172

Wittebole X, De Roock S, Opal SM (2013) A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence. doi:10.4161/viru.25991

Wright A, Hawkins CH, Änggård EE et al (2009) A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistent *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin Otolaryngol 34:349–357

5.2 Technical requirements for bacteriophages used as medicinal products (Study 9)

5.2.1 Quality and safety requirements for sustainable bacteriophage therapy products

J.P. Pirnay, B.G. Blasdel, L. Debarbieux, A. Buckling, N. Chanishvili, J.R. Clark, S. Corte-Real, L. Bretaudeau, A. Dublanchet, D. De Vos, J. Gabard, M. Garcia, M. Goderdzishvili, A. Górski, J. Hardcastle, I. Huys, E. Kutter, R. Lavigne, M. Merabishvili, E. Olchawa, K.J. Parikka, O. Patey, F. Pouilot, G. Resch, C. Rohde, J. Scheres, M. Skurnik, M. Vaneechoutte, L. Van Parys, G. Verbeken, M. Zizi, G. Van den Eede

Pharm Res. 2015; DOI 10.1007/sl l095-014-1617-7

International scientific journal, peer-reviewed

5.2.1 Quality and safety requirements for sustainable bacteriophage therapy products

J.P. Pirnay, B.G. Blasdel, L. Debarbieux, A. Buckling, N. Chanishvili, J.R. Clark, S. Corte-Real, L. Bretaudeau, A. Dublanchet, D. De Vos, J. Gabard, M. Garcia, M. Goderdzishvili, A. Górski, J. Hardcastle, I. Huys, E. Kutter, R. Lavigne, M. Merabishvili, E. Olchawa, K.J. Parikka, O. Patey, F. Pouilot, G. Resch, C. Rohde, J. Scheres, M. Skurnik, M. Vaneechoutte, L. Van Parys, G. Verbeken, M. Zizi, G. Van den Eede

Pharm Res. 2015; DOI 10.1007/sI I095-014-1617-7

International scientific journal, peer-reviewed

Pharm Res
DOI 10.1007/s11095-014-1617-7

PERSPECTIVE

Quality and Safety Requirements for Sustainable Phage Therapy Products

Jean-Paul Pirnay • Bob G. Blasdel • Laurent Bretaudeau • Angus Buckling • Nina Chanishvili • Jason R. Clark • Sofia Corte-Real • Laurent Debarbieux • Alain Dublanchet • Daniel De Vos • Jérôme Gabard • Miguel Garcia • Marina Goderdzishvili • Andrzej Górski • John Hardcastle • Isabelle Huys • Elizabeth Kutter • Rob Lavigne • Maia Merabishvili • Ewa Olchawa • Kaarle J. Parikka • Olivier Patey • Flavie Pouilot • Gregory Resch • Christine Rohde • Jacques Scheres • Mikael Skurnik • Mario Vaneechoutte • Luc Van Parys • Gilbert Verbeken • Martin Zizi • Guy Van den Eede

Received: 2 December 2014 / Accepted: 30 December 2014

ABSTRACT The worldwide antibiotic crisis has led to a renewed interest in phage therapy. Since time immemorial phages control bacterial populations on Earth. Potent lytic phages against bacterial pathogens can be isolated from the environment or selected from a collection in a matter of days. In addition, phages have the capacity to rapidly overcome bacterial resistances, which will inevitably emerge. To maximally exploit these advantage phages have over conventional drugs such as antibiotics, it is important that sustainable phage products are not submitted to the conventional long medicinal product development and licensing pathway. There is a need for an adapted framework, including realistic production and quality and safety requirements, that allowsa timely supplying of phage therapy products for 'personalized therapy' or for public health or medical emergencies. This paper enumerates all phage therapy product related quality

J.-P. Pirnay (✉) • D. De Vos • M. Merabishvili • G. Verbeken
Laboratory for Molecular and Cellular Technology, Queen Astrid Military Hospital, Bruynstraat 1, 1120 Brussel, Belgium
e-mail: jean-paul.pirnay@mil.be

B. G. Blasdel • R. Lavigne
Laboratory of Gene Technology, KU Leuven, 3001 Leuven, Belgium

L. Bretaudeau
Clean Cells SAS, Parc d'Activités Vendee Sud Loire, 85600 Bouffere, France

A. Buckling
Department of Biosciences, University of Exeter, Penryn TR10 9EZ, UK

N. Chanishvili • M. Goderdzishvili • M. Merabishvili
George Eliava Institute of Bacteriophages, Microbiology and Virology, 0160 Tbilisi, Georgia

J. R. Clark • J. Hardcastle
Novolytics Limited, Daresbury Science and Innovation Campus, Warrington WA4 4AD, UK

S. Corte-Real • M. Garcia
TechnoPhage SA, Edifício Egas Moniz, 1649-028 Lisbon, Portugal

L. Debarbieux
Department of Microbiology, Molecular Biology of the Gene in Extremophiles Unit, Institut Pasteur, 75724 Paris, France

A. Dublanchet • O. Patey
Service de Médecine Interne, Maladies Infectieuses et Tropicales, Centre Hospitalier Intercommunal, 94195 Villeneuve Saint-Georges, France

J. Gabard • F. Pouilot
Pherecydes Pharma, 93230 Romainville, France

A. Górski
Institute of Immunology & Experimental Therapy, Polish Academy of Sciences, 53-114 Wrocław, Poland

A. Górski
The Medical University of Warsaw, 02-006 Warsaw, Poland

I. Huys
Clinical Pharmacology and Pharmacotherapy, KU Leuven, 3000 Leuven, Belgium

E. Kutter
The Evergreen State College, Olympia, Washington 98505, USA

M. Merabishvili • M. Vaneechoutte
Laboratory for Bacteriology Research, Faculty of Medicine, Ghent University, 9000 Ghent, Belgium

E. Olchawa
Instytut Biotechnologii Surowic I Szczepionek Biomed S. A., 30-224 Kraków, Poland

K. J. Parikka • M. Zizi
Belgian Defense Staff, Medical Operational Command (COMOPSMED) Quartier Reine Elizabeth, 1200 Brussel, Belgium

K. J. Parikka • M. Zizi
Department of Physiology, Free University Brussels, 1090 Brussel, Belgium

Published online: 14 January 2015

and safety risks known to the authors, as well as the tests that can be performed to minimize these risks, only to the extent needed to protect the patients and to allow and advance responsible phage therapy and research.

KEY WORDS antibiotic resistance · medicinal product · phage therapy · production · quality and safety

Antimicrobial resistance in bacteria is an increasingly serious threat in every part of the world [1]. Without action, the world could be heading towards a post-antibiotic era in which common infections become fatal and currently routine surgeries become impossible. New initiatives to tackle the problem of antibiotic resistance are urgently needed.

One promising solution is the therapeutic use of bacteriophages – the viruses of bacteria, also known as phages – to treat bacterial infections. When discovered in the early twentieth century, phages were immediately applied in medicine (phage therapy) with variable success. After World War II, Western industry and policymakers preferred antibiotics, which at the time had obvious advantages in terms of breadth of coverage and ease of production and patentability, and phage therapy was pushed into the background. Today, phage therapy is again put forward as a potential way to address the current antibiotic crisis [2, 3].

Since time immemorial, phages have controlled bacterial populations on our planet, locked in an evolutionary arms race with their hosts (consisting of the repeated emergence of new phage infectivity and bacterial defense mutations). The capacity of bacteriophages to rapidly overcome bacterial resistance makes them suitable for flexible therapeutic applications. To maximally exploit this key advantage of phages over conventional 'static' drugs such as traditional small molecule-type antibiotics, it is important that sustainable phage products are not submitted to the conventional long medicinal product development and licensing pathway [4]. A key goal for the modern phage therapy community must be the development and validation of an expedited product development and licensing pathway in consultation with policymakers and competent authorities.

Georgian and Polish phage therapy centers maintain extensive therapeutic phage collections, which are regularly enriched with new phages, thus widening the total host range of the collection and adapting the collection to changing bacterial populations (with regard to host range and antibiotic resistance as well as phage resistance). Moreover, the effectiveness of phages can be readily improved by *in vitro* selection of (natural) phage mutants that exhibit an increased infectivity range. For example, it is possible to obtain potent lytic phages against problematic enteroaggregative *Escherichia coli* strains by isolation of new phages from the environment or by selection and adaptation of phages from an existing collection, and this often in a matter of days [5]. As such, phages could probably have been used to help control the O104:H4 (hybrid EAggEC STEC/VTEC pathotype) *E. coli* outbreak that caused the death of more than 50 patients in Germany in 2011. Unfortunately, authorized use of phages would not have been possible in this otherwise feasible context because under the existing medicinal product legislation such an anti-O104:H4 phage preparation would have taken years to develop, produce and register. Since phages are species and often even strain-specific, it is very likely that current O104:H4 specific phage preparations will not be effective against future epidemic enteroaggregative *E. coli* strains. 'Broad spectrum' phage cocktails active against bacteria that are likely to cause problems in the future could be developed in advance and used as a first line treatment for acute healthcare problems (*e.g.*, foodborne disease outbreaks and bacterial bioweapon threats). However, we need to keep in mind that some of these cocktails will not always work due to the greater biodiversity outside of the laboratory and the existing resistance to specific phages. The cocktails that initially work will need to be regularly updated (*e.g.*, supplemented with new phages in response to the evolution of phage resistance and the involvement of new circulating bacterial strains). There are indications that bacterial resistance to phages, even to cocktails containing multiple potent phages, will inevitably occur [6].

G. Resch
Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland

C. Rohde
Department of Microorganisms, Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, 38124 Braunschweig, Germany

J. Scheres
National Institute of Public Health NIZP, 00-791 Warsaw, Poland

J. Scheres
European Centre for Disease Prevention and Control (ECDC) Management Board, 171 83 Stockholm, Sweden

M. Skurnik
Haartman Institute, Department of Bacteriology and Immunology, and Research Programs Unit, Immunobiology, University of Helsinki, 00014 Helsinki, Finland

M. Skurnik
Helsinki University Central Hospital Laboratory Diagnostics, Helsinki Finland

L. Van Parys
5th Element of Medical Intervention (5 EMI), 1400 Nijvel, Belgium

G. Van den Eede
European Commission Joint Research Centre, Scientific Policy and Stakeholder Relations, 1049 Brussel, Belgium

Table I Expert Consensus Quality and Safety Requirements for Sustainable Phage Therapy Products

A. Production environment

When production activities include the processing of intermediate, bulk or finished phage products exposed to the environment, this must take place in an environment with specified air quality and cleanliness in order to minimize the risk of contamination. The effectiveness of these measures must be validated and monitored. Where intermediate, bulk or finished products are exposed to the environment during processing, without a subsequent microbial inactivation process, an *air quality* with particle counts and microbial colony counts equivalent to those of Grade A as defined in the current European Guide to Good Manufacturing Practice (GMP), Annex 1 and Directive 2003/94/EC is required with a background environment at least equivalent to GMP Grade D in terms of particles and microbial counts. The biosafety level (BSL) is determined by the host bacteria used in the production processes (*e.g.,* BSL-2 for *Pseudomonas aeruginosa*).

B. Production processes, equipment and materials

All equipment and material must be designed and maintained to suit its intended purpose and must minimize any hazard to recipients and staff. All critical equipment and technical devices must be identified and validated, regularly inspected and preventively maintained in accordance with the manufacturers' instructions. Where equipment or materials affect critical processing or storage parameters (*e.g.,* temperature, pressure, particle counts, microbial contamination levels), they must be identified and must be the subject of appropriate monitoring, alerts, alarms and corrective action, as required, to detect malfunctions and defects and to ensure that the critical parameters are maintained within acceptable limits at all times. All equipment with a critical measuring function must be calibrated against a traceable standard if available. Maintenance, servicing, cleaning, disinfection and sanitation of all critical equipment must be performed regularly and recorded accordingly.

Production processes must be described in detail (equipment, materials, culture media, additives, culture conditions, purification steps,..) in standard operating procedures (SOPs) and must be validated (procedures published in relevant peer-reviewed journals could be considered 'validated').

SOPs must detail the specifications for all critical materials and reagents. In particular, specifications for culture media, additives (*e.g.,* solutions) and packaging materials must be defined. Critical reagents and materials must meet documented requirements and specifications and when applicable the requirements of Council Directive 93/42/EEC of 14 June 1993 concerning medical devices and Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. If possible, animal component free culture media and additives should be used (the Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents *via* Human and Veterinary Medicinal Products (EMEA/410/01) in its current version is to be applied). If animal–product free media are not used, Transmitting Animal Spongiform Encephalopathy (TSE)-free certification should be obtained for all components containing products of animal origin.

Analytical methods can be validated according to: a) EMEA/CHMP/EWP/192217/2009 "Guideline on bioanalytical method validation" or b) CPMP/ICH /381/95 "ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology".

Bacteria and phage bank systems need to be set up. These bank systems typically consist of Master seed lots and Working seed lots. The generation and characterization of the banks should be performed in accordance with principles of CPMP/ICH guideline Q5D. The banked phages and bacteria should be characterized for relevant phenotypic and genotypic markers so that the identity, viability (activity for phages), and purity of organisms used for the production are ensured. Biological Resource Centers [10] could function as repositories for bacteriophage Master Seeds and host bacteria.

C. Quality Assurance and Quality Control (QA/QC) specifications

Products/characteristics	Control test	Limits of acceptance	Recommended test procedures
C.1. Host bacteria used in production (stock suspensions)			
The bacterial hosts used in the production process – with the exception of selection, adaptation and efficiency of plating (EOP) and host range determination – should be as safe (or least pathogenic) as feasible.			
Origin	Document pedigree/ history/pathogenicity level	Known origin	Screening of scientific literature, lab books, consignment letters,..
Identification	Identification at the species and strain levels	Matching species and strain identification	• State of the art clinical microbiology techniques • Highly discriminating (molecular/ genomic) typing techniques (*e.g.,* MLST, AFLP, PFGE, Rep-PCR,..)
Most often it will not be possible to find or quickly generate a suitable host bacterium that is free of prophages or phage-like elements, but one should nevertheless strive to use non-lysogenic strains, containing as few phages or other phage-like elements of genetic exchange [11, 12] as possible	• Induction of phages • Host genome screening for phage or phage-like elements	As few spontaneously produced (or by induction) temperate phages, complete prophage sequences or phage-like elements as possible[a]	• *In vitro* induction methods (Mitomycine C [13] or UV induction) • State of the art DNA sequencing and analysis (bioinformatics) procedures
Avoid mutator strains as host bacteria	Screen for mutator strains in case of doubt	No mutator strain	State of the art tests (*e.g.,* fosfomycin and rifampicin Disk Diffusion Tests) [14]
Validated preservation/storage (cryopreservation, freeze-drying,..)	Monitor storage conditions (*e.g.,* temperature)	Variable, depending on the preservation method	Variable (*e.g.,* temperature probes, temperature indicator labels,..)
C.2. Bacteriophages (Master Seed lots)			
Origin		• Known origin	

Table I (continued)

	Document bacteriophage pedigree/history (*e.g.*, isolation source)		Screening of scientific literature, lab books, consignment letters,…
		• Natural or naturally evolved bacteriophages	
Identification	• Identification at the family (subfamily), genus and species and strain level • Morphology and biology	Matching identification, morphology and biology	• State of the art DNA or RNA sequencing and analysis procedures • Highly discriminating genotyping techniques (*e.g.*, AFLP, fRFLP [15])[b] • State of the art classification according to the International Committee on Taxonomy of Viruses (ICTV) • State of the art electron microscopy (optional)[c] • One step growth curve [16]
Not containing potentially damaging genetic determinants (*e.g.*, conferring toxicity, virulence, lysogeny or antibiotic resistance)	Genome analysis for known potentially damaging genetic determinants	Absence of potentially damaging genetic determinants[d]	• State of the art DNA or RNA sequencing and genome analysis (bioinformatics) procedures
Non-transducing (optional) [17]	Screen for 'general transduction'	Does not pack random host DNA in a portion of progeny phage particles[e]	Transduction assay [18]
In vitro efficacy	Determination of host range on a panel of target species (reference) strains	Broad host range (if possible) Variable threshold according to species (*e.g.*, >75% for *Staphylococcus aureus*)	• Titration of bacteriophages against target bacteria according to the soft-agar overlay method [19] • Spot test [16]
	Stability of lysis (optional)[f]	Stable lysis in broth culture for 24–48 h	Appelmans method [20]
	Efficiency of plating (EOP) under conditions similar to eventual clinical application (optional)	Threshold EOP value	EOP determination [19]
	Determination of frequency of emergence of phage-resistant bacteria	Low frequency of emergence of resistance	Method described by Adams [19]
Improvement / adaptation / 'training' (if warranted)	Optimization of host range	Broadened and stable host range	• Titration of bacteriophages against target bacteria according to the soft-agar overlay method [16] • Spot test [16]
Validated preservation/storage (cryopreservation, freeze-drying,..)	Monitor storage conditions (*e.g.*, temperature)	Variable, depending on the preservation method	Variable (*e.g.*, temperature probes, temperature indicator labels,..)
C.3. Bacteriophages (Working Seed lots/Active Substances)			
Quantitative determination of active substance (bacteriophages)	Bacteriophage titration	Variable. Typically log(8) – log(10) plaque forming units (pfu)/ml	Soft-agar overlay method [19]
Identification of active substance	Genomic fingerprinting	Matching genomic fingerprint (max. deviation depends on method)	State of the art genotyping techniques (*e.g.*, AFLP, fRFLP [15])
Microbial contamination	Sterility (when there is no sense of urgency)[g]	Sterile (absence of micro-organisms)	Membrane filtration method based on the European Pharmacopoeia (EP)
	Absence of pathogens (when there is a sense of urgency)	Aseptic (absence of pathogens)	State of the art clinical microbiology methods
Toxicity	Bacterial endotoxin or lipopolysaccharides (LPS) quantification [21]	Depends on posology and method and route of administration. The maximum level for intravenous applications for pharmaceutical and biological products is set to 5 endotoxin units per kg of body weight per hour (EP).	Limulus Amebocyte Lysate (LAL) assay according to the EP (*e.g.*, kinetic-QCL method)
Bacterial DNA contamination[h]	Screen for (potentially damaging) host bacterial DNA	Absence of potentially damaging genetic determinants that are known to be present in the host bacterium	Methods for the quantification of bacterial DNA in general (*e.g.*, PicoGreen) or for the quantification

Table I (continued)

			of known DNA sequences (*e.g.,* qPCR)[i]
Acidity or basicity of aqueous solution	pH measurement	Variable (typically 6,5–7,5)	pH test (EP method)
Purity	Clarity of phage solution	Absence of visible particles	EP method, CPMP-ICH guideline
Validated preservation/storage (cooling, cryopreservation, freeze-drying,..)	Monitor/record/ demonstrate storage conditions (temperature,..)	Variable (*e.g.,* 2–8°C)	Variable (*e.g.,* temperature probes, temperature indicator labels,..)
C.4. Finished products			
Bulk products may be diluted (typically to log(5)–log(7) pfu/ml), combined or added to a carrier (hydrogel, ointment, cream, bandage,..) prior to clinical use. Dilution solutions, carriers and packaging materials must meet documented requirements and specifications and when applicable the requirements of Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. Carriers must be chosen that allow the required phage activity during the intended application period (stability).			
The following information must be provided either on the label or in accompanying documentation: (a) description (definition) and, if relevant, dimensions of the bacteriophage product; (b) date of production of the bacteriophage product (c) storage recommendations; (d) instructions for opening the container, package, and any required manipulation/reconstitution; (e) expiration dates (incl. after opening/manipulation); (f) instructions for reporting serious adverse reactions and/ or events; (g) presence of potentially harmful residues (*e.g.,* antibiotics, ethylene oxide); (h) contraindications; (e) how to dispose of unused (expired) bacteriophage products.			
Validated storage (cold storage,..)	Monitor/record/ demonstrate storage conditions (temperature,..)	Variable (*e.g.,* 2–8°C)	Variable (*e.g.,* temperature probes, temperature indicator labels,..)
D. Shelf life of phage stock suspensions, working solutions and finished products (at recommended storage conditions)			
Stability	• Periodic quantitative determination of the active substances (bacteriophages) or breakdown products • Periodic determination of sterility • Periodic pH measurements	The shelf life is the time period during which the product remains sterile and the activity and pH remain within specified limit thresholds	• Soft-agar overlay method [15] • CPMP-ICH guideline, Q5C, Q1A • Membrane filtration method (EP method) • pH test (EP method)
E. Surveillance			
The clinical use of phage therapy products must be surveyed and reported, including possible adverse events and reactions associated with the use of phage therapy products. A centralized (publicly available) reporting system is warranted.			

[a] Today it may be impossible to successfully cure some host strains that are indispensable for the production of some therapeutically interesting phages. In addition, in some cases it might be necessary to use phages that were isolated from the patient's bacteria and that are not able to replicate in known host strains devoid of prophages. However, since that sort of phage preparations are only designed to be used in that given patient, any remaining traces of DNA from that host bacterium would be orders of magnitude less than the amount already present in the patient from whom that bacterium was isolated for this purpose

[b] This genetic fingerprint can be used to timely identify bacteriophages and confirm their presence in Working Seed lots and in finished products, without having to re-perform full genome sequencing. It is however expected that fast, low-cost and accurate full genome sequencing and analysis (of bacteriophages) will replace routine microbial genotyping techniques in the near future

[c] In some cases (*e.g.,* novel bacteriophages with no homology in databases), electron microscopy could provide important information and could thus be warranted

[d] In general, it is recommended to only use lytic phages (and no temperate phages) in phage therapy. Lytic phages are more potent killers of host bacteria, making them more effective in therapy than temperate phages. Following lysogenic induction, temperate phages may transfer fragments of host bacterial DNA into non-targeted bacteria (possibly belonging to other species). This phenomenon is called transduction or phage-mediated horizontal gene transfer (HGT). If these DNA fragments contain toxin-encoding or antibiotic resistance-mediating genes, temperate phages could thus produce new pathogenic strains. However, in the future, the dogma that the use, in treatment, of temperate phages is impossible or undesirable because of the danger of HGT might be abandoned in certain circumstances (science- and risk-based decision, taking into consideration the patients' needs). In certain bacterial species, the number of strictly virulent phages is small and it might not be possible to isolate adequate new virulent phages in due time. Phage mediated HGT is abundant and virtually ubiquitous in bacterial populations and the additional and immediate danger to the patient related to the use of temperate phages in the course of phage therapy (days) is bound to be limited. Moreover, if a temperate phage acts as a lytic phage in relation to a particular pathogen, the probability of HGT might not be higher than for inherent genetic virulent phages [22]. In the future, temperate phages might specifically be used in therapy, *e.g.,* to introduce, by lysogenization, genes conferring sensitivity to antimicrobials [23] or to inhibit virulence traits [24]. Finally, antibiotic stress was also shown to induce genetic transformability in human pathogens [25]

[e] Today, it is not feasible to exclude the possibility of low levels of generalized transduction by therapeutic phages into any of the infecting and commensal bacteria present in or on the patient. The use in phage therapy of phages that mediate some random general transduction might be considered in certain circumstances (science- and risk-based decision, taking into consideration the patients' needs)

[f] In some cases, phages that produce stable lysis will not be found in a timely fashion. Phages that induce relatively fast *in vitro* bacterial resistance might then be considered

[g] In some cases, sterility may not be required (*e.g.,* 'non-sterile for topical application')

[h] Working Seed lots can be contaminated with low levels of DNA derived from the host bacteria used in production. Potentially damaging genetic determinants (*e.g.,* conferring toxicity, virulence or antibiotic resistance) might then be transferred (through transformation) to bacteria present in or on the patient, which could potentially make them (more) pathogenic. While this would be expected to occur at a level well below exchanges already going on within the patient's body involving their own pathogenic bacteria and phages already resident it makes sense to select hosts that are as devoid of pathogenicity factors as reasonably possible for growing therapeutic phage and treating the phage with DNase in the course of their purification to destroy such contaminants. If no non-pathogenic bacterial strain is available for growing the phage, constructing a 'defanged' host strain, with all pathogenicity determinants deleted, could be envisaged as the best main step in avoiding this issue. Note that the use of non-pathogenic host bacterial strains also reduces the potential hazard to the personnel involved in the production of therapeutic phages

[i] A threshold level should be determined. Note that some DNA quantification methods might also pick up phage DNA

Notwithstanding the Intellectual Property (IP) and regulatory hurdles, as well as the empirical evidence suggesting that stable and widely distributed phage preparations (*prêt-à-porter*) will need to be constantly updated, a few companies have picked up the gauntlet and are slowly moving along the elaborate and expensive conventional medicinal product licensing pathway. The development and marketing of phage medicinal products in the EU – including Good Manufacturing Practice (GMP) production, preclinical and Phase I, II and III clinical trials and centralized marketing authorization – is in fact technically possible (and indeed advisable for some products), providing some minor modifications and logical exemptions are made.

However, multiple discussions between experts, competent authorities and policymakers have led to an increasing awareness that *sustainable* (*sur-mesure*) phage therapy is not compatible with the conventional approaches to the development and application of medicinal products [4]. Next to the classical medicinal product pathway, which should be adjusted to support the industrial production of (first line) broad-spectrum phage cocktails or phage-derived products (*e.g.,* phage endolysins), there is a need for a specific framework (including realistic production and quality and safety requirements) that allows a timely (rapid) supplying of adapted productions of natural bacteriophages for 'personalized therapy'. This regulatory framework could be based on the Quality by Design (QbD) concept, which is increasingly applied to the development and production of biopharmaceutical molecules [7]. The QbD approach entails designing quality into the process and the product, and this in a science- and risk-based manner. Understanding patients' needs and determining the specific science and quality characteristics of the product that are linked to safety and efficacy are crucial components of QbD. More research is urgently needed to gather the required data with regard to the efficacy of phage therapy and to broaden our understanding of bacteria-phage coevolution in nature and in the context of human disease [8, 9]. To avoid the mistakes of the past (which lead to the current antibiotic resistance crisis), phage therapy products should not exclusively be developed and marketed as antibiotics, *i.e.,* applying current pharmacoeconomic principles. Ideally, phage therapy should be coordinated and standardized (in a first instance) by national phage therapy centers, which operate under the supervision of relevant public health authorities and in interaction with private stakeholders.

There are precedents for such a dedicated 'non-medicinal product' approach. In the European Union (EU), human tissues and cells that are not considered as 'Advanced Therapy Medicinal Products (ATMPs)' are procured, processed, tested and allocated by (or under the responsibility of) dedicated tissue establishments and are exclusively regulated by the EU Tissue and Cell Directives (EUTCDs). The EUTCDs consist of three Directives, the parent Directive (2004/23/EC), which provides the framework legislation, and two technical Directives (2006/17/EC and 2006/86/EC), which provide the detailed requirements of the EUTCD. The purpose of these Directives was to facilitate a safer and easier exchange of human tissues and cells between member states and to improve safety standards for European citizens. They set a benchmark for the standards that must be met when carrying out any activity involving tissues and cells for human application.

In view of further meetings with phage experts and representatives of the competent authorities and policymakers – coordinated by the European Commission Joint Research Centre, which acts in an advisory capacity to the Commission and its policy making directorates general –, a group of 'phage experts' (the authors of this paper) were asked through the intermediary of a not-for-profit organization (www.p-h-a-g-e.org) to set realistic quality and safety requirements for sustainable phage therapy products (Table I). These requirements are intended to apply to the production of phage therapy products (finished products), starting from banked characterized natural therapeutic bacteriophages (Master Seed lots), and possibly using intermediate bacteriophage products (Working Seed lots or Active Substances). They were roughly based on the EUTCD quality and safety standards for human cells and were

defined by consensus among 32 phage experts (biologists, geneticists, bioengineers, quality managers, pharmacists and MDs) from 12 countries. This document enumerates all possible phage product related quality and safety risks known to the experts, as well as the tests that can be performed to minimize these risks, only to the extent needed to protect the patients and to allow and advance responsible phage therapy and research. The exact tests used and limits applied will depend on the route of administration (*e.g.*, topical or systemic) and the regulatory path the product is being used under. These requirements do not address efficacy aspects of phage therapy products.

Should bacteriophages be used for a public health or medical emergency and no adequate finished products, Master Seed lots or Working Seed lots are available, then less stringent requirements could be considered, pending compliance (as quick as possible) to the quality and safety requirements.

ACKNOWLEDGMENTS AND DISCLOSURES

We would like to thank Marc Struelens [European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden] for his critical comments.

Several authors are employed by Small and Medium Enterprises that are developing phage therapy products.

REFERENCES

1. Antimicrobial resistance: Global report on Surveillance. Geneva, World Health Organization (WHO), 2014. Available from: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1 (accessed 18/09/14).
2. Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, Huovinen P, *et al.* Tackling antibiotic resistance. Nat Rev Microbiol. 2011;9:894–6.
3. Borysowski J, Międzybrodzki R, Górski A. Phage therapy: current research and application. UK: Caister Academic Press; 2014.
4. Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, *et al.* The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm Res. 2011;28:934–7.
5. Merabishvili M, De Vos D, Verbeken G, Kropinski AM, Vandenheuvel D, Lavigne R, *et al.* Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. PLoS ONE. 2012;7:e52709.
6. Hall AR, De Vos D, Friman VP, Pirnay JP, Buckling A. Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa in vitro* and in wax moth larvae. Appl Environ Microbiol. 2012;78:5646–52.
7. Verbeken G, Pirnay JP, De Vos D, Jennes S, Zizi M, Lavigne R, *et al.* Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine. Arch Immunol Ther Exp (Warsz). 2012;60:161–72.
8. Seed KD, Yen M, Shapiro BJ, Hilaire IJ, Charles RC, Teng JE, *et al.* Evolutionary consequences of intra-patient phage predation on microbial populations. Elife. 2014;3:e03497.
9. Díaz-Muñoz SL, Koskella B. Bacteria-phage interactions in natural environments. Adv Appl Microbiol. 2014;89:135–83.
10. OECD. Best practice guidelines for biological resource centers – quality management, biosecurity, building capacity, preservation of biological resources, data management. Paris: Secretary-General of the OECD; 2007.
11. Lang AS, Zhaxybayeva O, Beatty JT. Gene transfer agents: phage-like elements of genetic exchange. Nat Rev Microbiol. 2012;10:472–82.
12. Christie GE, Dokland T. Pirates of the Caudovirales. Virology. 2012;434:210–21.
13. Burlage RS, Atlas R, Stahl D, Geesey G, Sayler G. Techniques in microbial ecology. USA: Oxford University Press; 1998.
14. Galán JC, Tato M, Baquero MR, Turrientes C, Baquero F, Martinez JL. Fosfomycin and rifampin disk diffusion tests for detection of *Escherichia coli* mutator strains. J Clin Microbiol. 2004;42:4310–2.
15. Merabishvili M, Verhelst R, Glonti T, Chanishvili N, Krylov V, Cuvelier C, *et al.* Digitized fluorescent RFLP analysis (fRFLP) as a universal method for comparing genomes of culturable dsDNA viruses: application to bacteriophages. Res Microbiol. 2007;158:572–81.
16. Kutter E, Sulakvelidze A. Bacteriophages: Biology and application. Boca Raton: CRC Press; 2005.
17. Matilla MA, Fang X, Salmond GP. Vulnalikeviruses are environmentally common agents of horizontal gene transfer in pathogens and biocontrol bacteria. ISME J. 2014;8:2143–7.
18. Petty NK, Foulds IJ, Pradel E, Ewbank JJ, Salmond GPC. A generalized transducing phage (φIF3) for the genomically sequenced *Serratia marcescens* strain Db11: a tool for functional genomics of an opportunistic human pathogen. Microbiol. 2006;152:1701–8.
19. Adams MH. Bacteriophages. New York: Interscience Publishers New York; 1959.
20. Appelmans R. Le dosage du bactériophage. Compt Rend Soc Biol. 1921;85:1098.
21. Parracho HMRT, Burrowes BH, Enright MC, McConville ML, Harper DR. The role of regulated clinical trials in the development of bacteriophage therapeutics. J Mol Genet Med. 2012;6:279–86.
22. Krylov V, Shaburova O, Krylov S, Pleteneva E. A genetic approach to the development of new therapeutic phages to fight *Pseudomonas aeruginosa* in wound infections. Viruses. 2013;5:15–53.
23. Edgar R, Friedman N, Molshanski-Mor S, Qimron U. Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. Appl Environ Microbiol. 2012;78:744–51.
24. Chung IY, Sim N, Cho YH. Antibacterial efficacy of temperate phage-mediated inhibition of bacterial group motilities. Antimicrob Agents Chemother. 2012;56:5612–7.
25. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. Science. 2006;313:89–92.

5.3 Validation of the proposed regulatory framework for bacteriophage therapy (Study 10)

5.3.1 Key issues in bacteriophage therapy: a report of a dedicated workshop at the Viruses of Microbes II meeting

I. Huys, M. Vaneechoutte, G. Verbeken, L. Debarbieux

Research in Microbiology. 2013; 164:806-810

International scientific journal, peer-reviewed

5.3.1 Key issues in bacteriophage therapy: a report of a dedicated workshop at the Viruses of Microbes II meeting

I. Huys, M. Vaneechoutte, G. Verbeken, L. Debarbieux

Research in Microbiology. 2013; 164:806-810

International scientific journal, peer-reviewed

ELSEVIER

Institut Pasteur

Research in Microbiology 164 (2013) 806–810

www.elsevier.com/locate/resmic

Meeting report

Key issues in phage therapy: a report of a dedicated workshop at the viruses of microbes II meeting

1. Summary

A workshop addressing key issues in phage therapy was organized by the non-profit organization P.H.A.G.E. during the Viruses of Microbes II meeting, held in July 2012 in the Royal Military Academy, Brussels, Belgium. This report summarizes the major points that were addressed by the panel of experts from the academic, public, private and legal domains.

2. The experts

During the Viruses of Microbes II meeting, held in July 2012 at the Royal Military Academy in Brussels, Belgium, a workshop was dedicated to the clinical and regulatory barriers and opportunities of the development of phage therapy in Europe. A panel, chaired by **Isabelle Huys** (Univ. Leuven, Belgium) and **Martin Zizi** (Free Univ. Brussels, Belgium, and president of P.H.A.G.E.), included experts from diverse domains: **Jacques Scheres**, board member of the European Centers for Disease Control (ECDC, Stockholm, Sweden), a leading European authority on the surveillance of bacterial resistance problems and new antibacterial strategies. **Jérôme Larché**, head of the intensive care unit in Narbonne Hospital (France) and president of the non-profit organization, PHAGESPOIR, who represented the public sector. **Harald Brüssow** from the Nestlé Research Center, Vers-Chez-Les-Blancs, Switzerland and **David Harper** from BioControl, AmpliPhiBiosciences (Colworth Science Park, London, UK) who were delegates from private companies. H. Brüssow is involved since years in directing phage research and safety trials (e.g. Brüttin and Brüssow, 2005) for a large multinational company (Nestlé). D. Harper is the founder and scientific officer of the startup which carried out the first phage trials using modern, rigorous, clinical trials standards (Wright et al., 2009). **Andrzej Górski** from the Hirszfeld Institute of Immunology and Experimental Therapy (Wroclaw, Poland) represented the long-standing 'Eastern Bacteriophage therapy tradition' and is leading translational and fundamental research on phages (Górski et al., 2009, 2012). **Angus Buckling** from the University of Exeter (UK) has published extensively on the interaction of phages and bacteria to better understand and deal with the problems of emerging bacterial resistance to phages when they are used therapeutically as antibacterial agents (Hall et al., 2012). **Gilbert Verbeken** (Laboratory of Molecular and Cellular Technology, Burn Wound Center, Queen Astrid Military Hospital, Brussels, Belgium) is a specialist concerned with the numerous regulatory hurdles confronting clinical trials of phage therapy (Verbeken et al., 2007). **Isabelle Huys**'s expertise is in intellectual property (IP) affairs particularly focused in phage therapy.

3. The need for phage therapy

The discussion started with what is, without a doubt, considered as one of the most serious problems confronting public health authorities: the emergence of bacterial pathogens that are resistant to the existing antibiotics and the failure to develop new types of antibiotics. There was unanimity that the overuse of antibiotics has to be reduced in order to slow down the emergence of pan-resistant bacterial pathogens and in addition, physicians as well as medical students need to be educated accordingly. However, other antibacterial solutions are urgently needed since some patients are nowadays confronted with the difficulty if not yet impossibility to treat their bacterial infections.

Phage therapy, initiated early in the previous century by Félix D'Herelle at the Institut Pasteur (Paris, France), and almost exclusively expanded post World War II to several Eastern European countries, represents a sustainable solution, as demonstrated by its clinical use especially in Poland and Georgia. In Western Europe, phage therapy does not picture the conventional therapeutic schemes for infectious diseases. Nevertheless, would it be unreasonable to consider unconventional solutions when dealing with therapeutic impasses involving the life and well-being of patients? A reasonable, responsible and appropriate response to that question, proposed along this workshop, was: 'Surely not, if done with moderation and reason, and keeping in consideration that the only objective is the improvement of the patient's condition.'

4. The eastern legacy

Phage therapy has been implemented for decennia under the umbrella of the Declaration of Helsinki, a non-binding legal instrument providing for some ethical principles

0923-2508/$ - see front matter
http://dx.doi.org/10.1016/j.resmic.2013.03.020

regarding human experimentation. A large set of data on phage therapy has via this way partially been published previously (Górski et al., 2009; Weber-Dabrowska et al., 2000). In addition, under the auspices of the UK Global Threat Reduction Program, a literature review and an English translation of some of the past Georgian clinical trials have been published as well (Chanishvili, 2009, 2012).

The question is now whether it would be useful to preserve and exploit such valuable experimental data from the past and how such data could be implemented to serve the community. There was general agreement that preservation of this abundance of data from the East is of real importance and requires for example the creation of a centralized bank, or an online inventory of the various Phage Collections. When possible, associated useful scientific and clinical information should be collected. Such a central facility would enable the community to classify, organize and study those phages and most importantly would increase their availability to be employed for therapeutic use. This would also prevent isolation of related phages again and again.

5. Assessing phage efficacy

The ability of phages to act as anti-infectious agents (efficacy) is most probably due to their direct killing action on bacteria. This is most prominent in treatments of acute infections resulting from massive clonal bacterial invasion, as is the case for *Escherichia coli* enteritis in cattle, while *Salmonella* and *Campylobacter* infections, being more dispersed, would be less prone to phage treatments. Complete elimination of the pathogen is however not required, as the reduction of the bacterial load appears sufficient to decrease symptoms, allowing the immune defense to take over. For human treatments, what really matters is the clinical outcome, the favorable clinical progression, not the complete eradication of the harmful bacteria. This was observed in the chronic otitis clinical trials conducted by BioControl, where the symptoms were relieved for several patients despite the persistence of some *Pseudomonas aeruginosa* (Wright et al., 2009).

Although counterintuitive, there might be good scientific reasons for such observations. Besides a favorable initial strong reduction in bacteria following phage application, the resistant bacterial mutants against the phages that eventually emerge may be less virulent and then less prone to elimination by the immune system. Indeed, this loss of virulence is frequently due to mutations affecting ligands involved in bacterial invasion (adhesins, invasins) or subversion of the immune system (PAMPs). Such ligands exposed to the cell surface are also used by phages as receptors for adsorption and the subsequent infection of their bacterial hosts. In a classical example described 30 years ago, *E. coli* K1, a virulent encapsulated strain, acquired resistance during an experimental treatment with phages in animals by becoming K1-negative as a consequence of mutations and lost its virulence (Smith and Huggins, 1982).

Another possible explanation of the ability of phages to act as anti-infectious agents, that is not related to their antibacterial activity, might be their immunomodulatory and anti-inflammatory properties, which thus far have remained understudied (Górski et al., 2012).

6. The problem of resistance

Although bacterial resistance to phage could cause a simultaneous decrease in bacterial virulence (see above), much caution needs to be exerted to avoid the same problems that we now experience with antibiotics. Widespread use of phages for human therapy, extensive prescriptions of phage products and the treatment of livestock could inevitably lead to substantial shedding of these phage strains in the environment and thus could result in increased bacterial resistance to these phages. It will be therefore important, at least during sometime, to keep phage applications on a limited scale, for clear-prescribed indications. Education of future medical doctors toward responsible prescription of phages should also be included in the development of phage therapy. Resistance is however from a different kind as with antibiotics. There is the continuously ongoing arms race/competition between bacteria and phages, and specific phages that are able to infect the formerly resistant bacterial strains can be expected to quickly emerge (Pirnay et al., 2012). More experimental evolution studies are however necessary to determine the potential negative evolutionary consequences of unlimited phage therapy. Ideally, environmental microbiology studies could help to address this potential problem before it became a serious concern. Last but certainly not the least aspect of the development of phage therapy comes from the use of phages in combination with conventional antibiotic treatments, as supported by a synergy observed *in vitro* between some phages and some antibiotics (Comeau et al., 2007). Most likely, some clinical data from Eastern Europe dataset should exist and be used to guide this strategy that has been so far under looked *in vivo*.

7. Regulatory hurdles

Accepting phage therapy as new cure in Western Europe involves crucial legal issues which seem very phage-specific. Therefore, some propose to regulate phage therapy via a separate, eventually new regulatory category that would account for the intrinsic ability of phages to evolve over time, as opposed to traditional antibiotics (Verbeken et al., 2007; Pirnay et al., 2011). The existence of such a specific regulatory category could significantly facilitate clinical studies. There are various examples of existing discrepancies in the ways regulatory authorities treat different classes of products. For example, a separate category has been created to allow the toleration of homeopathic products, because the authorities were confronted with a fait accompli of an enormous variety of products already established in the market. Hence, they were forced to create a separate category to assure at least a bare minimum control of these products. On the other hand,

some consider phages to be categorized as 'biologics', fitting in the same subcategory than vaccines. This subcategory is clearly defined, in part, as 'aimed at curing diseases with living viruses', a definition which fits phages perfectly well. Moreover, the vaccine subcategory tolerates some modest level of naturally occurring changes of the vaccine products – without the necessity of initiating an entirely new regulatory procedure. For example, when protection from a newly arisen variant pathogen is urgently required (as in a flu vaccine), the appropriate modification of the vaccine can be easily and rapidly accommodated by the flexibility of the regulations. Such flexibility would be invaluable for therapeutic phage cocktails, since they will need to be regularly updated because of the emergence of phage resistant bacterial mutants. However, phage cocktails and phage-based products in general may actually require greater compositional flexibility than the once-in-a-year updates that suffices for vaccines. Then the question remains whether regulators would accept to increase further the current flexibility.

An additional difficulty to consider is the standardization of the therapeutic phage-based products. Even a product made from a complete clonal phage population can change within the patient. While this can be seen as the ultimate advantage of phage therapy over treatment with antibiotics, as the phage can co-adapt to an evolving pathogen, this intrinsic genetic variability poses serious problems with respect to current policies advocated by the regulatory authorities that place major emphasis on the compositional uniformity of a therapeutic product.

Phage therapy requires the selection and application of phages specifically targeting a particular patient's bacterial strain. In such a setting, it is important to distinguish between two regulatory definitions, namely 'product quality' and 'production quality'. For companies that want to market a well-defined product, regulatory instances require 'product quality'. However, for custom-made products such as, for example, the different combinations of phages that may be required to deal with an emerging bacterial pathogen, it might be better to opt for 'production quality' rather than 'product quality'. Thus, although the product may change slightly, the production procedure would be standardized to ensure uniform high quality of each individual batch.

Another way out of this regulatory conundrum is to get a 'hospital exemption' from the regulatory restrictions in case phage therapy is applied within a hospital under supervision of a medical doctor in a patient-specific setting. This would allow the therapeutic application of phages, in cases of urgent and serious health problems, like the German Shiga-toxin producing EHEC strains that caused 54 deaths in June 2011, and which could not be successfully treated with antibiotics. Such tolerance would be necessary also today to offer a solution for some chronically infected patients for which antibiotics are not sufficient any more.

In addition to regulatory hurdles, issues with respect to IP have withheld companies and other investors from investing into phage therapy thus far. There are several patents (primarily originating from the US) claiming protection for various aspects of phage therapy, ranging from natural phages, to phage cocktails and to various phage methods of treating infectious diseases of bacterial origin. However, the validity and enforceability of these patents are dubious since many of them are overly broad and/or are imprecisely defined. As a consequence, there is a widespread issue of legal uncertainty for companies, hospitals and public institutions involved in the applications of phage therapy.

8. And then, there is safety

Despite the sometimes kafkaesque regulations and exorbitant safety concerns about phage therapy, the fact remains that it has been generally proven to be very safe. However, there simply is no way to avoid some safety regulations for phage therapy. Indeed, however strong the claims regarding general safety of phage preparations employed in the Eastern European experience are, from the rigid point of view of the regulatory authorities, they absolutely need to be convinced of the safety of the precise product that will be employed in any future therapy trials. For example, they must be convinced of the absence of any possible harmful contamination or any possible risk coming from the phage itself or its solute. Previous safety data records, no matter how convincing and numerous they may be, do not constitute a credible assurance of the safety of a related new product (or even of a different batch of exactly the same product) used in clinical trials. This is not a specific problem related to phage therapy, even for well-established products in clinical trials (for example, magisterial preparations that have been long employed in a hospital), the regulatory authorities require that investigational medical products documents be filed and that certification be provided that these products are produced under Good Manufacturing Procedures conditions. Although phages seem safe and can cause spectacular improvements in patients in some situations, this therapy still needs to be applied with some caution. In spite of the substantial empirical use in Eastern Europe, the numerous experimental animal studies, the encouraging preclinical studies on *ex vivo* samples, rigorous scientific evaluation will still be necessary for general clinical use of phage therapy.

9. High costs, an inescapable major hindrance in the pursuit of clinical trials

The best way to rigorously evaluate phage therapy is through clinical trials in order to establish proof of principle and to stimulate additional funding. But, in the first place, funding itself is the primary bottleneck for setting up expensive clinical trials. Hence, the current lack of clinical trials on phage therapy indicates a general hesitancy of industry to invest the large sums necessary to confirm phage safety and efficacy. Presumably, and in view of the regulatory and IP hindrances indicated above, the current evaluation of the industry is that it is not worth the large risks for them to do it by themselves. Perhaps with the help of public support they may get involved.

It is actually quite striking that public scientific institutions have begun to promote 'translational' and 'personalized' medicines as a widespread concepts, but that many of the

creative ideas provided by the publically funded scientists for potentially useful therapeutic agents and therapy strategies are too often put aside when facing regulatory requirements. The huge development cost of such long-term research projects and the inevitable uncertainties presented by the strict regulatory requirements cannot be carried alone by academic institutions, neither industrial startups. In consequence, the notion that the development of phage therapy will be comparatively cheap in comparison to new antibiotic development is probably too simplistic and fundamentally flawed. Considering the cost of production, both strategies are relatively inexpensive but they are also equivalently expensive to be developed for use in clinical trials and to eventually bring into the market, because the costs of overcoming the regulatory restrictions (e.g. number of trials, patients to be included, …) are essentially the same for both and, indeed, very expensive. Moreover, since a phage preparation may have only a narrow spectrum of sensitive bacterial targets, the return-on-investment for each preparation often may be too low for any company to justify the risk. As clinical trial costs for phages are at least as high as they are for antibiotics and since the market for each phage product could be intrinsically rather limited in their narrow therapeutic range, the profitability for private phage-producing companies may simply be too modest to provide economic sustainability.

10. The European authorities

The lack of implementation of phage therapy presents a real concern and triggers interest at the highest levels in Europe, such as the ECDC which is responsible for the surveillance of infectious diseases. This interest might forecast a significant evolution in Europe's attitude toward phage therapy, which in the past can be fairly described as highly skeptical. The time has now come in Europe, to take phage therapy seriously. Patient groups and medical doctors should be encouraged to exert pressure on the both European institutions and elected political bodies because policy changes are only rarely initiated from above. More often these structures only respond positively and rapidly to initiatives that originate from extramural sources that have wide public support. Since regulators only apply rules, they do not change them, political pressure must be exerted from the bottom to influence the top. This strategy is essentially what is being applied in France by PHAGESPOIR, by actively implicating the patients who would be the potential beneficiaries for phage therapy in the appeals to the regulatory authorities and politicians. The responsibility to move forward and not tolerate the status quo rests not just with the public health authorities and health professionals, but also squarely on the patients. There is also an urgent need for research funding to explore and improve the efficacy of phage therapy and importantly to unambiguously demonstrate its safety.

One could then see explicitly with a video presentation made by His Excellency Mr. **Pieter De Crem**, Belgian Minister of Defense (http://player.vimeo.com/video/43203048) a glimpse of the involvement of public authorities as a sign that the status quo may not stand any longer.

In conclusion, this workshop was perceived by both the audience and the panel members as a milestone in the development of phage therapy (Brüssow, 2012). The road will certainly not be short, as normal with development of new medical treatments. But it is a road that must be explored and that hopefully can get to its end before an inevitable outbreak of a lethal infectious disease caused by an emergent bacterium, resistant to our existing arsenal of antibiotics, emerges.

Acknowledgment

We thank the organization team of the meeting VoM II as well as the board of PHAGE more specifically to have organized this workshop. This work was supported with a research community grant (WO.022.09) of the FWO Vlaanderen.

References

Brüttin, A., Brüssow, H., 2005. Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob. Agents Chemother. 49, 2874–2878.

Brüssow, H., 2012. What is needed for phage therapy to become a reality in Western medicine? Virology http://dx.doi.org/10.1016/j.virol.2012.09.015. pii: S0042-6822(12)00456-4.

Chanishvili, N., 2009. A Literature Review of the Practical Application of Bacteriophage Research. Eliava Institute of Bacteriophage, Microbiology & Virology, Tbilisi, Georgia, p. 184.

Chanishvili, N., 2012. Phage therapy – history from Twort and d'Herelle through Soviet experience to current approaches. Adv. Virus Res. 83, 3–40.

Comeau, A.M., Tétart, F., Trojet, S.N., Prère, M.-F., Krisch, H.M., 2007. Phage-antibiotic synergy (PAS): beta-lactam and quinolone antibiotics stimulate virulent phage growth. PLoS One 2 (8), e799.

Górski, A., Miedzybrodzki, R., Borysowski, J., Weber-Dabrowska, B., Lobocka, M., Fortuna, W., Letkiewicz, S., Zimecki, M., Filby, G., 2009. Bacteriophage therapy for the treatment of infections. Curr. Opin. Investig. Drugs 10, 766–774.

Górski, A., Międzybrodzki, R., Borysowski, J., Dąbrowska, K., Wierzbicki, P., Ohams, M., Korczak-Kowalska, G., Olszowska-Zaremba, N., Lusiak-Szelachowska, M., Kłak, M., Jończyk, E., Kaniuga, E., Gołaś, A., Purchla, S., Weber-Dąbrowska, B., Letkiewicz, S., Fortuna, W., Szufnarowski, K., Pawełczyk, Z., Rogóż, P., Kłosowska, D., 2012. Phage as a modulator of immune responses: practical implications for phage therapy. Adv. Virus Res. 83, 41–71.

Hall, A.R., De Vos, D., Friman, V.P., Pirnay, J.P., Buckling, A., 2012 Jun 1. Effects of sequential and simultaneous application of bacteriophages on populations of *Pseudomonas aeruginosa* in vitro and in waxmoth larvae. Appl. Environ. Microbiol.. (Epub ahead of print).

PHAGESPOIR. http://phagespoirs.unblog.fr/.

P.H.A.G.E.: Phage for Human Application Group Europe.http://www.p-h-a-g-e.org.

Pirnay, J.P., De Vos, D., Verbeken, G., Merabishvili, M., Chanishvili, N., Vaneechoutte, M., Zizi, M., Laire, G., Lavigne, R., Huys, I., Van den Mooter, G., Buckling, A., Debarbieux, L., Pouillot, F., Azeredo, J., Kutter, E., Dublanchet, A., Górski, A., Adamia, R., 2011. The phage therapy paradigm: Prêt-à-porter or sur-mesure? Pharm. Res. 28, 934–937.

Pirnay, J.P., Verbeken, G., Rose, T., Jennes, S., Zizi, M., Huys, I., Lavigne, R., Merabishvili, M., Vaneechoutte, M., Buckling, A., De Vos, D., 2012. Introducing yesterday's phage therapy in today's medicine. Future Virol. 7 (4), 379–390.

Smith, H.W., Huggins, M.B., 1982. Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol. 128, 307–318.

Verbeken, G., De Vos, D., Vaneechoutte, M., Merabishvili, M., Zizi, M., Pirnay, J.P., 2007. European regulatory conundrum of phage therapy. Future Microbiol. 2, 485–491.

Weber-Dabrowska, B., Mulczyk, M., Górski, A., 2000. Bacteriophage therapy of bacterial infections: an update of our institute's experience. Arch. Immunol. Ther. Exp. 48, 547–551.

Wright, A., Hawkins, C.H., Anggård, E.E., Harper, D.R., 2009. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. Clin. Otolaryngol. 34, 349–357.

Isabelle Huys*
Centre for Pharmaceutical Care and Pharmaco-Economy, Faculty of Pharmaceutical Sciences, Univ. Leuven, Leuven, Belgium

Mario Vaneechoutte
Laboratory Bacteriology Research (LBR), Faculty of Medicine & Health Sciences, Univ. Ghent, Belgium

Gilbert Verbeken
Burn Wound Centre, Queen Astrid Military Hospital, Brussels, Belgium

Laurent Debarbieux
Department of Microbiology, Institut Pasteur, F-75015 Paris, France

*Corresponding author.
E-mail address: isabelle.huys@pharm.kuleuven.be

6 Stakeholders moral responsibility in relation to bacteriophage therapy

6.1 Investigation of moral principles related to bacteriophage therapy (Study 11)

6.1 Investigation of moral principles related to bacteriophage therapy (Study 11)

6.1.1 Taking bacteriophage therapy seriously: a moral argument

G. Verbeken, I. Huys, J.P. Pirnay, S. Jennes, N. Chanishvili, J. Scheres, A. Górski, D. De Vos, C. Ceulemans

BioMed Research International. 2014; 24: Article ID 621316, 8 pages

International scientific journal, peer-reviewed

6.1.1 Taking bacteriophage therapy seriously: a moral argument

G. Verbeken, I. Huys, J.P. Pirnay, S. Jennes, N. Chanishvili, J. Scheres, A. Górski, D. De Vos, C. Ceulemans

BioMed Research International. 2014; 24: Article ID 621316, 8 pages

International scientific journal, peer-reviewed

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 621316, 8 pages
http://dx.doi.org/10.1155/2014/621316

Research Article

Taking Bacteriophage Therapy Seriously: A Moral Argument

Gilbert Verbeken,[1,2,3] Isabelle Huys,[2,4] Jean-Paul Pirnay,[1] Serge Jennes,[5] Nina Chanishvili,[6] Jacques Scheres,[7,8,9] Andrzej Górski,[10,11] Daniel De Vos,[1] and Carl Ceulemans[3]

[1] *Laboratory for Molecular and Cellular Technology, Burn Wound Centre, Queen Astrid Military Hospital, Bruynstraat 1, 1120 Brussel, Belgium*
[2] *Faculty of Pharmaceutical and Pharmacological Sciences, KU Leuven, Herestraat 49, 3000 Leuven, Belgium*
[3] *Department of Behavioural Sciences, Royal Military Academy, Renaissancelaan 30, 1000 Brussel, Belgium*
[4] *Centre for Intellectual Property Rights, KU Leuven, Minderbroedersstraat 5, 3000 Leuven, Belgium*
[5] *Burn Wound Centre, Queen Astrid Military Hospital, Bruynstraat 1, 1120 Brussel, Belgium*
[6] *R&D Department, Eliava Institute of Bacteriophage, Microbiology and Virology, 3 Gotua Street, 0160 Tbilisi, Georgia*
[7] *University Hospital Maastricht, Maastricht University Medical Centre, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands*
[8] *Dept. Hospital Organization, National Institute of Public Health NIZP, ul. Chocimska 24, 00-791 Warsaw, Poland*
[9] *Dept. Medical Microbiology, University Medical Centre Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*
[10] *Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. Rudolfa Weigla 12, 53-114 Wrocław, Poland*
[11] *The Medical University of Warsaw, Żwirki i Wigury 61, 02-091 Warsaw, Poland*

Correspondence should be addressed to Carl Ceulemans; carl.ceulemans@rma.ac.be

Received 20 January 2014; Revised 14 April 2014; Accepted 21 April 2014; Published 29 April 2014

Academic Editor: Carla Carvalho

The excessive and improper use of antibiotics has led to an increasing incidence of bacterial resistance. In Europe the yearly number of infections caused by multidrug resistant bacteria is more than 400.000, each year resulting in 25.000 attributable deaths. Few new antibiotics are in the pipeline of the pharmaceutical industry. Early in the 20th century, bacteriophages were described as entities that can control bacterial populations. Although bacteriophage therapy was developed and practiced in Europe and the former Soviet republics, the use of bacteriophages in clinical setting was neglected in Western Europe since the introduction of traditional antibiotics. Given the worldwide antibiotic crisis there is now a growing interest in making bacteriophage therapy available for use in modern western medicine. Despite the growing interest, access to bacteriophage therapy remains highly problematic. In this paper, we argue that the current state of affairs is morally unacceptable and that all stakeholders (pharmaceutical industry, competent authorities, lawmakers, regulators, and politicians) have the moral duty and the shared responsibility towards making bacteriophage therapy urgently available for all patients in need.

1. Introduction

1.1. Factual (Nonnormative) Observations concerning Bacteriophage Therapy. The excessive and improper use of antibiotics has led to an increasing incidence of bacterial resistance and a significant threat to human health [1, 2]. Yearly, more than 400.000 people are infected by multidrug resistant bacterial strains, often called "superbugs" [3]. Superbugs have a considerable economic impact: extra hospital costs and related productivity losses amount to more than 1.5 billion Euros per year within the European Union (EU). In the United States (US), infections with multidrug resistant bacteria cause 20 billion US$ in additional health care costs and 35 billion US$ societal costs annually [4].

At the same time, it is becoming more and more difficult and expensive to develop new antibiotics as an adequate response to the phenomenon of multidrug resistance. Actually, very few new antibiotics are in the pipeline of the pharmaceutical industry at the moment [5].

Early in the 20th century, bacteriophages were described as entities that can control bacterial populations. Bacteriophages (or "phages") are viruses that infect and replicate

within specific bacteria without harming others. Although bacteriophage therapy (hereafter BPT) was developed and practiced in Europe and the former Soviet republics, the western world abandoned the use. This was mainly because at that time (1930s) there was a lack of knowledge of what a bacteriophage really was (a virus) as well as the discovery of antibiotics. These molecules are well-characterized chemical substances, relatively easy to produce in a well-controlled fashion, initiating the golden age of antibiotics, the so called "miracle drugs" [6].

Given the worldwide antibiotic crisis, the existing and continued experiences build up in Eastern Europe and the former Soviet Republics combined with recent encouraging animal and human study results; there is a growing interest for BPT in modern medicine and the agrobioindustry, a recognized potential reservoir for antibiotic resistant germs [1, 3, 7–10].

Despite this growing interest, introducing BPT in the western medical world remains highly problematic as a consequence of *four main obstacles*. First, historical clinical data about the safety and effectiveness of BPT are not considered proven and validated by European regulators. Second, given the substantial costs and investment in the development and marketing of conventional medicinal products by the pharmaceutical industry, there is in our actual pharmacoeconomic model an imperative demand for a strong intellectual property (IP) protection. For now, such protection is rather fragile for natural lytic phages. Third, an efficient and effective BPT-concept needs to be flexible and tailored to the patient [11, 12]. That requirement is not compatible with the usual timeframes (years) for the development and the marketing of conventional medicinal products [11, 12]. Rather, the regulatory framework for medicinal product development, as present in most countries, calls for drugs to have a fixed chemical composition. Bacteriophages challenge this definition by being mutable. Last (fourth obstacle), uncertainty exists about the potential negative coevolutionary consequences of unlimited use of BPT [13]. In view of these obstacles, access to BPT for patients in need remains highly problematic as discussed earlier [12, 14].

Recently, Henein emphasized that so far no bioethical bacteriophage therapy debate has been published. He gave the most recent *E. coli* 0104 outbreak as an example [15]. Indeed in 2011, an emerging strain of O104:H4 *Escherichia coli* caused a serious outbreak of food borne haemolytic uremic syndrome and bloody diarrhoea in Germany. Antibiotics were of questionable use and 54 deaths occurred, beside tens of clinical cases with lasting sequels [16, 17]. Several bacteriophage research groups had in their collection isolated candidate therapeutic bacteriophages that efficiently lyse the *E. coli* O104:H4 outbreak strain [18–20]. The public health sector never asked for these phages during the outbreak and none of the scientific papers published during the outbreak mentioned BPT as a potential treatment. Nestlé Research Centre even offered their phage isolate to the German public health sector during the epidemic, but the proposal was apparently not addressed [20].

Międzybrodzki et al. [21] addressed briefly the ethical aspects of bacterial drug resistance and phage therapy. The authors also highlighted the appeals for decisive changes in the policies governing the development of antimicrobials. Bacteriophages should be considered as a public good and the government should be responsible for their development and production. Thus, the development and introduction of new antimicrobials should not only be regulated by market forces [21].

The main purpose of this paper is not so much to fleece out these four main obstacles, nor to determine how an adequate regulatory framework for BPT might look like. This has already been done in other publications [11, 12, 14, 22]. We here argue why there exists a moral need or duty to develop such a regulatory framework. The different actors in the field, mainly the industrial partners, politicians and regulators as well as consumers, urgently need to take up their responsibility in order to guarantee BPT accessibility for patients in need. What is more, when the costs of phage therapy and antibiotic therapy were compared, phages were approximately 50% cheaper than antibiotics. This means that a wider application of phage therapy could lead to substantial savings in healthcare costs and make antibacterial therapy accessible to those who otherwise cannot afford treatment [23].

1.2. Normative-Ethical Considerations concerning BPT. Given the above nonnormative specifications of BPT, the basic moral problem associated with BPT can be formulated as follows. BPT, when used in a flexible (tailor-made, locally developed) and sustainable manner, has the potential of saving thousands of lives every year [11, 24]. However, due to the above-mentioned obstacles, access to that therapy remains highly problematic in the western world. How can we argue from a moral point of view that this situation is simply unacceptable?

Central to this moral problem are the preservation and restoration of the health and well-being of the patient. Two basic underlying moral principles are relevant in this patient-centred approach and will be further investigated in this paper. The first is the *principle of nonmaleficence,* which implies the obligation not to inflict harm on another. This principle is designed to protect the patient. The second principle is the *principle of beneficence,* which implies the obligation to prevent or to remove harm or the obligation to promote good [25].

2. Bacteriophage Therapy: An Ethically Justified Medical Therapy?

The fact that efficacy of BPT has not yet been proven according to European regulatory standards is one of the obstacles that clearly suggests the moral relevance to investigate the *principle of nonmaleficence* in the context of BPT. Indeed, a patient has a right not to be subjected to a medical treatment or therapy that has not yet been rigorously tested for its effectiveness and possible health risks (as is BPT). The moral duty, however, not to subject a patient to a not yet approved therapy is not an absolute, but a *prima facie duty*. This means that under well-defined, specific circumstances this duty not

Table 1: Six criteria for a therapy to be labelled as an EJMT.

It is morally permissible to set aside the *prima facie duty* not to impose a risk of harm if and only if
(i) there is a just cause;
(ii) those who want to put aside the duty not to impose a risk of harm have good intentions;
(iii) there is a reasonable chance that the just cause will be realized;
(iv) the harm prevented will outweigh the risk of harm imposed;
(v) the just cause cannot be obtained with at least the same probability of success but without imposing a risk of harm;
(vi) those who decide on putting aside the duty not to impose a risk of harm constitute a legitimate authority.

to subject someone to a not yet approved therapy can be set aside [26]. At this point in the argument, it is appropriate to introduce the notion of an "*Ethically Justified Medical Therapy*" (EJMT). This is a medical therapy that has not yet obtained an official approval for its health effectiveness (at least not according to western standards), and/or for which some doubts remain concerning possible health risks, but the use of which seems to be morally acceptable given the specific circumstances of the case at hand. For such a therapy to be labelled as an EJMT, six criteria need to be met (Table 1) [27].

First of all, there has to be a *just cause* or a very good reason for subjecting a patient to a possibly hazardous medical therapy and/or a therapy the effectiveness of which has not yet been demonstrated in a rigorous manner. The moral weight of whatever it is we want to achieve with this therapy has to be sufficiently important. In the case of BPT-therapy we might think of a patient whose life or limb is threatened by a serious bacteriological infection. Saving that patient's limb or threatened life, for instance, constitutes such a good reason beyond any doubt.

Secondly, we need to make sure that all the moral agents involved are motivated by *ethically proper intentions*. In a clinician-patient relationship, the medical practitioner ought to have the intention to help the patient in need, although other interests (e.g., hospital-related) may play a certain role as well. Therefore, if indeed a patient's health is at stake (a good reason), then our intention for using the yet unapproved therapy has to be about improving the patient's health condition and not about commercial, research, or cost-reducing benefits.

Next, there needs to be a *reasonable chance* that the use of the therapy in a particular case will have the desired result. In case of BPT, based on preliminary examinations and testing by experienced specialists, multiple case studies reporting success were published [28–30].

Efficacy of bacteriophage therapy has not yet been proven according to European regulators, but anyway the concept of reasonable chance does not imply real proof of such efficacy. What is more, there must be a good prospect that the probable health benefits will outweigh the risks of subjecting the patient to the therapy (*proportionality*). It is important in this respect to try to avoid as much as possible undesired side effects related to BPT [31–33].

Another criterion is that the unapproved therapy needs to be a *last resort*. All existing treatments must have been tried with little or no success. BPT might for instance be considered as such a last resort when marketed antibiotics are no longer effective. In reality, in particular circumstances of multidrug resistance, BPT offers today a *reasonable and feasible* alternative or complimentary treatment approach to save such patients. Indeed it has been shown that BPT and traditional antibiotic therapy can create a synergic beneficial effect [34, 35].

Medical practitioners' assessment in these circumstances is crucial. It needs to be stressed that in clear cases of antibiotic resistance, BPT most likely constitutes the first and not just the last resort. Indeed, the last resort principle only demands that we consider reasonable alternatives, since hopeless situations due to lack of adequate alternatives need to be avoided as much as possible according to international and national legislations and declarations. In Belgium, the compassionate use regime provides such a "last resort" mechanism, whereby a medical practitioner may apply an unapproved product provided marketing authorization is applied for or clinical trials are ongoing. At the international level, Article 37 of the Declaration of Helsinki states the following: *"In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available"* [36]. Note however, that the Declaration of Helsinki, although at the international medical community level is well accepted as a basic document, is not a national binding law, meaning that it has no national juridical value and as such could put the practitioner in a position of juridical vulnerability as experienced in France (personal communications, M.D. A. Dublanchet and Court Lawyer B. Papin).

Finally, a decision to subject a patient to a not yet approved therapy needs to be made in respect with the patient's right to autonomy (*legitimate authority*) [26]. However, it should be noted that a patient's consent to be subjected to a not yet approved treatment is not a sufficient condition to go ahead with the therapy. The other above-mentioned criteria need to be respected as well. Within this context, we need to determine whether a patient's (or its legal representative) consent constitutes a necessary condition.

Although these six criteria were originally developed within another context, namely, that of the just war theory

(JWT), it seems that these criteria can also be of valuable use in the ethical discussion on BPT [37]. This is because the underlying ethical argument is very similar. The JWT starts from the supposition that war is a moral evil, and that because of this we have an obligation to avoid war as much as possible. Notice here again that the obligation not to wage war is not an absolute, but a *prima facie duty*. In specific circumstances (determined by the above criteria) war can be a morally acceptable option (for instance in case of self-defense or humanitarian intervention). A very similar moral mechanism is at work in our discussion about BPT. Subjecting a patient to a not yet approved therapy is morally wrong, but sometimes, in specific (exceptional) circumstances (determined by the six criteria), it can be a morally *acceptable* option to subject a patient to such unapproved therapy, and not just a *morally excusable option*.

Whether or not BPT is an EJMT needs to be checked on a *case-by-case* basis. Numerous types of bacterial infections exist. Any manner to combat such infection needs to be considered separately. In fact, medical ethical committees provide also case-by-case reasoning while evaluating proposals for treatment.

We would like to conclude the first part of our argument by saying that the six criteria we used to evaluate BPT's moral permissibility form the ethical basis for some kind of *precautionary* regulatory framework. Until it can be adequately shown that BPT can live up to western health care standards, there will be a constant need to justify the use of BPT on a case-by-case basis. Moreover, given the specific nature of BPT (its interactive and ever-evolving character) and the need to focus on tailor-made solutions, it will be difficult, if not impossible, to claim once and for all (as it is probably the case for traditional "static" chemical medicinal products) that BPT is effective and safe. This would also imply that any regulatory framework designed to assure the safety and effectiveness of flexible and sustainable BPT will never completely lose its precautionary character.

3. Towards a Moral Duty to Invest in the Development of Bacteriophage Therapy

3.1. Do Pharmaceutical Companies Have a Duty to Care? Demonstrating that BPT constitutes an EJMT in a sufficient number of cases is of course but a first part of our moral argument. Showing that in a specific case BPT is an EJMT makes its use *morally permissible* in that specific case. But showing *moral permissibility* for BPT is only a *necessary condition* for our purpose, which is demonstrating that it is simply morally unacceptable to obstruct (or, at least, to not sufficiently facilitate) the accessibility to BPT. We also need to show that somehow there exists a *moral duty* to take the necessary steps in order to make flexible and sustainable BPT available in a more organized fashion.

Pharmaceutical companies are the primary actors in the business of developing new and improved medicinal products and therapies. Hospitals do not have the financial capacity and resources to fully develop bacteriophage-based products according to the current pharmaceutical medicinal product guidelines. Although our initial focus will be on pharmaceutical companies, this does not mean that other actors (like public authorities) are absolutely absolved of all responsibility in this respect. We will come back to this specific issue later on in this paper.

In our patient-centred approach, we perceived that the principle of *nonmaleficence* provides a so-called *prima facie* moral protection for the patient against therapies and treatments that have not yet proven their health effectiveness and/or for which some doubts remain concerning their possible health risks. In trying to establish a moral duty to contribute to the development of lifesaving therapies, such as BPT [28, 30, 38], it is suitable to turn to the other basic moral principle in bioethics, that of *beneficence*.

Despite the fact that it is not in the interest of the classic pharmaceutical industry, organized in accordance with the actual pharmacoeconomic environment, to invest in sustainable BPT (see the above mentioned obstacles), the beneficence-principle seems to provide a sufficient moral basis for arguing that the pharmaceutical industry has a duty to do so anyway.

In the 1970s, most supporters of market economy embraced Friedman's view that the social responsibility of business is to increase its profits, not to relax the conditions of profit-maximization on behalf of the wider interests of society [39]. But, is this acceptable when it comes to healthcare? Surely, companies involved in the healthcare industry should live up to their responsibilities towards the public interest, not only towards their shareholders. To quote Blasszauer: "*medicine is a moral enterprise whether it is practiced in the system of slavery or market economy*" [40]. Defenders of Friedman's thesis claim that for executives to use company resources to advance social goals, it would be for them to usurp the political function. In this context it might thus be up to the political world to demand healthcare companies to defy the laws of economics and fulfil social duties [12, 41].

One of the reasons why the above conclusion is not that straightforward has to do with the specific nature of the beneficence-principle. Indeed this basic principle is especially morally relevant within the relation between the health care professional and his patient. Because of his specific role, it can be said that the health care professional has the moral obligation to undertake all the necessary and reasonable measures to improve his patient's health condition (or to prevent his patient's health from deteriorating). The relational context between a patient and the pharmaceutical industry is of course very different from the one between the health care professional and his patient. It is no longer a relation of care, compassion, and beneficence, but a relation of an economic or a commercial kind. The industry's role is to develop, produce, and sell medicinal products, whereas the role of the patient is that of a consumer. This does not mean that the pharmaceutical industry has absolutely no obligations whatsoever towards its patient-consumers. The industry has the obligation to take all the necessary and reasonable measures to ensure the effectiveness and the safety of the medicinal products it decides to develop, produce, and sell [42]. Within that same commercial relation ("patient-consumer/pharmaceutical industry") it is very hard, as far

as we can tell, to justify the imposition of an additional requirement on the pharmaceutical industry to invest in the development and production of medicinal products that are of a lifesaving importance to some patients, but that are rather uninteresting from a pure commercial point of view as is the case for flexible and sustainable BPT that has been shown lifesaving in specific individual cases [28, 30, 38]. Here we need to look for another way to morally justify an obligation to make available for as many patients as possible medicinal products or therapies for which there does not seem to exist a sufficient commercial incentive to start a development and production process.

3.2. Do Pharmaceutical Companies Have a Social Responsibility to Invest in BPT in Order to Promote Overall Social Welfare? Perhaps a more promising line of argument is of a *utilitarian kind*. One might quite convincingly argue that economic actors in our society, like private businesses and companies, do not only have specific client-related obligations, but also a somewhat broader social responsibility to promote overall well-being in society. If we agree that this is the case (that there exists such a utilitarian-based responsibility), then it still remains to be seen of course whether the moral requirement to promote novel antimicrobial approaches like BPT can indeed be based on such a notion of social responsibility. To verify this, we need to explore two separate questions.

The *first question* goes as follows: to what extent may we assume that the promotion of BPT (eventually in combination with existing therapies) constitutes indeed the course of action that, compared to other possible alternatives, will lead to better results in confronting the health challenges related to an increasing antibiotic resistance problem in bacteria (hereafter the resistance problem)? Proving that BPT is the best option in tackling this specific health problem from a utilitarian point of view is essentially a *technical matter* that requires further thorough knowledge in microbiology and in health care economics. In other publications it has been shown that this may be the case [7, 43, 44].

Establishing with a reasonable degree of certainty that BPT is indeed a good option in confronting the resistance problem from a utilitarian point of view constitutes, however, a necessary, but not a sufficient condition for our purpose to demonstrate that there is such a thing as a moral requirement to promote BPT. A part of our common-sense morality clearly states that promoting the best overall result does not automatically generate a moral requirement to do so. We can illustrate this with an example. Suppose that ten people are on the verge of losing their lives (that they are about to drown). Suppose also that person A could save all of them if he wanted to. The only trouble is that person A can only do so with a considerable risk to himself. Although, saving the ten lives (even with the risk of losing his own life in the process) is the best option from a utilitarian point of view; there is a general agreement within common-sense morality that person A is under no moral obligation to do so. In this specific example person A is allowed to favour his own life, even if sacrificing it in order to save the ten others would be preferable from a utilitarian viewpoint. In the theory of normative ethics, common-sense morality contains so-called agent-favouring options (or agent-favouring prerogatives) [45]. These are moral principles that protect an agent from the obligation to *always* promote the overall good. The key word here is "always".

Indeed, in some cases the promotion of the overall good does create a moral requirement to do so. For instance: if person A could save the lives of those ten people with practically no risk for himself, he can no longer avoid the obligation to do so. In order to understand the mechanism of the agent-favouring options better, it is essential to take into account two factors: the (probable) cost to the agent, and the (probable) amount of overall good or well-being that is at stake. If the amount of good at stake outweighs the cost to the agent (who has no risk to lose his life), then the requirement to promote the overall good can no longer be blocked by the agent-favouring option. This seems to suggest that the option protecting a moral agent's self-interest is characterized by some kind of threshold, the level of which is determined by the cost the moral agent will have to pay when he decides to serve the overall good. Once the amount of good or well-being at stake crosses that threshold, the creation of a moral requirement to bring about this amount of good can no longer be blocked by the agent-favouring option. A general rule seems to be then: the higher the cost to the agent, the higher the threshold, the stronger the agent-favouring option, and the lesser the probability that the agent will be subjected to the obligation to promote the overall good. It should be clear, however, that whenever the interests of an agent are protected by such an agent-favouring option, he or she is still at liberty to promote the overall good. If, for instance, person A is a heroic kind of a person and he will not hesitate to save those ten people, even if this means sacrificing his own life. It is obvious that such an act will not be morally condemned. Far from it: such an act will typically be praised and admired. But again, it is not an *obligatory* act, but rather a *supererogatory* act inspired by idealism [46].

Let us assume for the sake of argument that such agent-favouring options are indeed a part of our common-sense morality. This being the case, it might very well be that the economic and commercial interests of the pharmaceutical industry are going to be protected by such options. Even if it would appear that promoting BPT is indeed the best choice in tackling the resistance problem, pharmaceutical enterprises cannot be morally obligated to sacrifice their own commercial interests in order to give priority to the development of BPT. It is possible, however, that some pharmaceutical companies could decide to go ahead anyway with the development of BPT. But, again, such a decision would constitute a supererogatory act, not a mandatory act.

We now come to our *second question*: is it reasonable to assume that there exists such an agent-favouring option that protects the private interests of the pharmaceutical industry and thereby blocks the creation of a moral requirement to promote BPT? Answering this question requires a more profound cost/benefit analysis. In case of pharmaceutical companies' investments in bacteriophage product development, costs are considerable since all regulatory processes need to be conducted [20]. Assuming that (a) cost-based

option(s) protecting the interests of a moral agent is (are) indeed a part or our common-sense morality and (b) the development of BPT will definitely entail a commercial costs to a pharmaceutical company (resources that will be invested in this kind of research and development can no longer be used to develop more lucrative products); it is also fair to assume that pharmaceutical companies will try to protect their private interests by appealing to such agent-favouring option and pretend that there is no such thing as a duty to promote BPT. Whether or not the pharmaceutical industry is justified in hiding behind such agent-favouring option will depend on two factors: what is the *moral force* of that option and will it be strong enough to block a moral requirement to promote BPT? At this point in the argument we will need to evaluate the importance of the cost to the pharmaceutical industry. This will indeed give us some idea of the moral force of the agent-favouring option (the higher the cost, the stronger the option). Today, any organization, pharmaceutical company, or any nonprofit actor like a hospital, willing to implement BPT, is expected to follow the classical marketing authorization and market placement procedures. The costs for developing BPT following these frameworks can be as much as 400–800 million USD [15] and not realistic for nonprofit actors.

In addition, intellectual property rights (IPRs) that in general provide owners exclusivities to recoup investment costs are hardly unavailable (or weak) in the context of BPT [15]. The reason is that IPRs do not protect natural products (e.g., natural bacteriophages) or processes covering natural phenomena (e.g., mechanism of action of BPT based on the inherent coevolution of bacteria and bacteriophages). However, given the widespread availability of bacteriophages and the fact that natural bacteriophages are from a structural point of view less complex than other biological (protein-based) products or advanced therapies medicinal products, the costs to develop BPT could eventually be lower compared with these latter medicinal products.

Will the overall health benefits of saving thousands of lives, generated by the introduction of BPT, cross the option's threshold (the height of which is determined by the option's moral force)? To verify this, we need to compare the costs of investment in BPT development by pharmaceutical companies with the unproven potential BPT of, for example, savings of the lives of 25.000 Europeans each year [28, 30, 38], with the cost associated with bacterial outbreaks caused by bacterial infections [47], costs to the social security for hospitalized patients [3], and emotional costs associated with the disease itself. According to the authors of this paper, these costs to society by not developing BPT clearly indicate that the option's threshold protecting the pharmaceutical industry's private interests will be crossed. In our opinion, pharmaceutical companies hence do have a moral duty to contribute in one or another way to the development of BPT. Thinking of phage therapy as a sustainable antibacterial approach should have the potential of cost reduction for society that should be considered as a major incentive for companies to invest in BPT. However, in the current regulatory climate, the pharmaceutical companies are unable to do it without adequate incentives or support. Clearly other stakeholders like the public authorities need to provide the right incentives by creating feasible regulatory frameworks.

4. Closing the Moral Gap

4.1. Reconciling Private Interests and Social Responsibilities. According to the authors, previous analysis demonstrates that arguments can be found to support a moral requirement for the pharmaceutical industry to promote BPT. It remains to be seen what specific form this moral requirement will take. The pharmaceutical industry, due to its specific knowledge and know-how, will certainly have some role to play in the validation process of the clinical data relating to BPT (obstacle (1)) and/or in furthering the research concerning the potential negative coevolutionary consequences of unlimited use of BFT (obstacle (4)). In addition, it is important to consider how in the future industrial companies will maintain (keep on respecting) this moral duty and how these companies can be made aware of the importance of creating and maintaining high moral standards in the long run. History portrays similar developments, more specific in the domain of cell and gene therapy. Several regulatory incentives, specific types of subsidies and models for public-private partnerships have contributed to stimulate the industry for investments into these therapies [48]. More in particular, a specific European framework covering advanced therapy medicinal product (Regulation 1394/2007) was created, offering legal incentives (e.g., scientific advice at reduced costs) to developers of cell and gene based therapies. Specific calls for research funding were launched via the FP7 programs of the European Commission, and large-scale collaborative efforts are in place between industry and academia, under the umbrella of, for example, Europe's Innovative Medicines Initiative (IMI).

4.2. Other Stakeholders' Responsibilities. Until now we have solely focused on the pharmaceutical industry as the principal bearer of this potential moral requirement to promote BPT. Obviously, political authorities, much more so than private companies, have a social responsibility to promote public health in the most efficient way they can. What is more, due to their public nature, public authorities cannot hide behind cost-based options to protect their "private" interests as private companies can. As such, since we have shown that according to the evidence available, the promotion of flexible (characterized by its locally produced and tailor-made nature) and sustainable BPT is, compared to other alternatives, for different particular disease situations, the best solution to promote overall health benefits, then there is a public moral duty to do so. This particular public requirement to promote this specific kind of BPT can perhaps best manifest itself by providing an adapted regulatory framework, so that the full potential of BPT as a locally prepared and tailor-made therapy can finally be realized.

5. Conclusion

In this paper, we have argued that the current state of affairs as described in the introduction is morally unacceptable.

We succeeded in underpinning the desirability for developing a flexible and sustainable BPT-adapted regulatory framework with the necessary moral force. The authors are aware that moral arguments in favour of BPT may equally be identified (via a similar moral analysis) and apply to other areas of drug development (e.g., orphan diseases). We argued that the pharmaceutical industry has a moral duty to invest in BPT in view of the social responsibility they need to take. But of equally crucial importance is the role of the competent public authorities to create the appropriate regulatory and legal framework to stimulate companies to invest in BPT. Political representatives and lawmakers have an inevitable, logical responsibility to support health care and welfare. That is what they are for. We identified a shared responsibility making BPT accessible for patients in need. The development and production of BPT products in a pharmaceutical context (clinical trials, production requirements, marketing authorization procedures...) requires time. Patients in need have no time. Therefore, lawmakers and regulators need to design appropriate solutions on a short term to buffer for the years needed for companies to develop BPT-based medicinal products. Much more urgent and optimal, regulatory solutions need to be created to allow hospitals to adopt patient-oriented and tailored BPT in a legal way for treating those patients that are waiting to be cured.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Gilbert Verbeken and Isabelle Huys are coshared.

References

[1] M. A. Cooper and D. Shlaes, "Fix the antibiotics pipeline," *Nature*, vol. 472, no. 7341, p. 32, 2011.

[2] M. Kutateladze and R. Adamia, "Bacteriophages as potential new therapeutics to replace or supplement antibiotics," *Trends in Biotechnology*, vol. 28, no. 12, pp. 591–595, 2010.

[3] K. Bush, P. Courvalin, G. Dantas et al., "Tackling antibiotic resistance," *Nature Reviews Microbiology*, vol. 9, no. 12, pp. 894–896, 2011.

[4] R. R. Roberts, B. Hota, L. Ahmad et al., "Hospital and societal costs of antimicrobial-resistant infections in a chicago teaching hospital: implications for antibiotic stewardship," *Clinical Infectious Diseases*, vol. 49, no. 8, pp. 1175–1184, 2009.

[5] C. Wattal, J. K. Oberoi, R. Raveendran, and K. J. Prasad, "Current status of newer antiinfectives," *Recent Patents on Anti-Infective Drug Discovery*, vol. 3, no. 3, pp. 206–224, 2008.

[6] J. Fernebro, "Fighting bacterial infections—future treatment options," *Drug Resistance Updates*, vol. 14, no. 2, pp. 125–139, 2011.

[7] D. Maura and L. Debarbieux, "Bacteriophages as twenty-first century antibacterial tools for food and medicine," *Applied Microbiology and Biotechnology*, vol. 90, no. 3, pp. 851–859, 2011.

[8] S. B. Levy and B. Marshall, "Antibacterial resistance worldwide: causes, challenges and responses," *Nature Medicine*, vol. 10, no. 12, pp. S122–S129, 2004.

[9] K. K. Kumarasamy, M. A. Toleman, T. R. Walsh et al., "Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study," *The Lancet Infectious Diseases*, vol. 10, no. 9, pp. 597–602, 2010.

[10] E. Brzuszkiewicz, A. Thürmer, J. Schuldes et al., "Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic Escherichia coli (EAHEC)," *Archives of Microbiology*, vol. 193, no. 12, pp. 883–891, 2011.

[11] J.-P. Pirnay, D. De Vos, G. Verbeken et al., "The phage therapy paradigm: prêt-à-porter or sur-mesure?" *Pharmaceutical Research*, vol. 28, no. 4, pp. 934–937, 2011.

[12] J.-P. Pirnay, G. Verbeken, T. Rose et al., "Introducing yesterday's phage therapy in today's medicine," *Future Virology*, vol. 7, no. 4, pp. 379–390, 2012.

[13] S. Meaden and B. Koskella, "Exploring the risks of phage application in the environment," *Frontiers in Microbiology*, vol. 4, article 358, 2013.

[14] G. Verbeken, J. P. Pirnay, D. De Vos et al., "Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine," *Archivum Immunologiae et Therapia Experimentalis*, vol. 60, no. 3, pp. 161–172, 2012.

[15] A. Henein, "What are the limitations on the wider therapeutic use of phage?" *Bacteriophage*, vol. 3, no. 2, Article ID e24872, 2013.

[16] C. Frank, D. Werber, J. P. Cramer et al., "Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany," *The New England Journal of Medicine*, vol. 365, no. 19, pp. 1771–1780, 2011.

[17] M. Michael, E. J. Elliott, G. F. Ridley, E. M. Hodson, and J. C. Craig, "Interventions for haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura," *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD003595, 2009.

[18] D. Maura, E. Morello, L. du Merle, P. Bomme, C. Le Bouguénec, and L. Debarbieux, "Intestinal colonization by enteroaggregative *Escherichia coli* supports long-term bacteriophage replication in mice," *Environmental Microbiology*, vol. 14, no. 8, pp. 1844–1854, 2012.

[19] M. Merabishvili, D. De Vos, G. Verbeken et al., "Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany," *PLoS One*, vol. 7, no. 12, Article ID e52709, 2012.

[20] H. Brussow, "What is needed for phage therapy to become a reality in Western medicine?" *Virology*, vol. 434, no. 2, pp. 138–142, 2012.

[21] R. Międzybrodzki, J. Borysowski, B. Weber-Dąbrowska et al., "Clinical aspects of phage therapy," *Advances in Virus Research*, vol. 83, pp. 73–121, 2012.

[22] I. Huys, M. Vaneechoutte, G. Verbeken, and L. Debarbieux, "Key issues in phage therapy: a report of a dedicated workshop at the viruses of microbes II meeting," *Research in Microbiology*, vol. 164, no. 7, pp. 806–810, 2013.

[23] R. Miedzybrodzki, W. Fortuna, B. Weber-Dabrowska, and A. Górski, "Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment," *Postepy Higieny i Medycyny Doświadczalnej*, vol. 61, pp. 461–465, 2007.

[24] L. B. Rice, "Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE," *Journal of Infectious Diseases*, vol. 197, no. 8, pp. 1079–1081, 2008.

[25] M. Munyaradzi, "Critical reflections on the principle of beneficence in biomedicine," *The Pan African Medical Journal*, vol. 11, article 29, 2012.

[26] S. Kagan, *Normative Ethics*, Westview Press, Boulder, Colo, USA, 1998.

[27] R. B. Miller, *Casuistry and Modern Ethics. A Poetics of Practical Reasoning*, University of Chicago Press, London, UK, 1996.

[28] E. Kutter, D. De Vos, G. Gvasalia et al., "Phage therapy in clinical practice: treatment of human infections," *Current Pharmaceutical Biotechnology*, vol. 11, no. 1, pp. 69–86, 2010.

[29] A. Khawaldeh, S. Morales, B. Dillon et al., "Bacteriophage therapy for refractory *Pseudomonas aeruginosa* urinary tract infection," *Journal of Medical Microbiology*, vol. 60, no. 11, pp. 1697–1700, 2011.

[30] N. Chanishvili, *A Literature Review on the Practical Application of Bacteriophage Research*, Nova Science Publishers, 2012.

[31] G. Verbeken, J. P. Pirnay, R. Lavigne et al., "Call for a dedicated European legal framework for bacteriophage therapy," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 62, no. 2, pp. 117–129, 2014.

[32] C. Loc-Carrillo and S. T. Abedon, "Pros and cons of phage therapy," *Bacteriophage*, vol. 1, no. 2, pp. 111–114, 2011.

[33] S. Hoe, D. D. Semler, and A. D. Goudie, "Respirable bacteriophages for the treatment of bacterial lung infections," *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, vol. 26, no. 6, pp. 317–335, 2013.

[34] A. M. Comeau, F. Tétart, S. N. Trojet, M.-F. Prère, and H. M. Krisch, "Phage-antibiotic synergy (PAS): β-lactam and quinolone antibiotics stimulate virulent phage growth," *PLoS One*, vol. 2, no. 8, article e799, 2007.

[35] A. E. Kirby, "Synergistic action of gentamicin and bacteriophage in a continuous culture population of *Staphylococcus aureus*," *PLoS One*, vol. 7, no. 11, Article ID e51017, 2012.

[36] Declaration of Helsinki, http://www.wma.net/en/30publications/10policies/b3/.

[37] B. Coppieters and N. Fotion, *Moral Constraints on War. Principles and Cases*, Lexington Books, Lanham, Md, USA, 2nd edition, 2008.

[38] http://www.mrsa-forum.com/products/index.asp?forumID=9270&subject=Phage-Therapy-Center-Saved-My-Life.

[39] M. Friedman, "The social responsibility of business is to increase its profit," *New York Times Magazine*, 1970.

[40] B. Blasszauer, "Petty corruption in health care," *Journal of Medical Ethics*, vol. 23, no. 3, pp. 133–134, 1997.

[41] J. P. Pirnay, A. Vanderkelen, N. Ectors et al., "Beware of the commercialization of human cells and tissues: situation in the European Union," *Cell Tissue Bank*, vol. 13, no. 3, pp. 487–498, 2012.

[42] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2001L0083:20110721:EN:PDF.

[43] N. Chanishvili, *A Literature Review of the Practical Application of Bacteriophage Research*, Nova Science Publishers, New York, NY, USA, 2012.

[44] S. T. Abedon, S. J. Kuhl, B. G. Blasdel, and E. M. Kutter, "Phage treatment of human infections," *Bacteriophage*, vol. 1, no. 2, pp. 66–85, 2011.

[45] F. M. Kamm, *Intricate Ethics, Rights, Responsibilities, and Permissible Harm*, Oxford University Press, Oxford, UK, 2007.

[46] T. L. Beauchamp and J. F. Childress, *Principles of Biomedical Ethics*, Oxford University Press, New York, NY, USA, 2009.

[47] M. Pirson, P. Leclercq, T. Jackson, M. Leclercq, M. Garrino, and C. Sion, "Financial consequences of hospital-acquired bacteraemia in three Belgian hospitals in 2003 and 2004," *Journal of Hospital Infection*, vol. 68, no. 1, pp. 9–16, 2008.

[48] H. Stevens, G. van Overwalle, B. E. J. van Looy, and I. Huys, "Perspectives and opportunities for precompetitive public-private partnerships in the biomedical sector," *Biotechnology Law Report*, vol. 32, no. 3, pp. 131–139, 2013.

7 Concluding discussion

7 Concluding discussion

7.1 Legislative frameworks

7.1.1 Options inside the Medicinal Products Directive 2001/83/EC: under or alongside the Biological Medicinal Products

Bacteriophages are not straightforward inanimate and stable substances [Chapter 2.1.2 / Chapter 2.3.2]. They are evolvable and natural biological entities. A sustainable bacteriophage therapy concept should fully acknowledge the potential of the co-evolutionary aspect of the bacteriophage/ bacterium couplet. Only then, it should be possible to put the inherent potential of bacteriophages as natural biological bacterium controllers to use [Chapter 3.1.2]. Indeed, bacteria will inevitably become resistant to bacteriophages, but due to the continuously ongoing evolutionary arms race between the two protagonists, specific bacteriophages that are able to infect the formerly resistant bacterial strains can be expected to quickly emerge.

The existing pharmaceutical regulatory framework and business models are not compatible with such a dynamic and at the long term sustainable bacteriophage therapy concept. Therefore, a suitable legislative environment for bacteriophage therapy should be developed [Chapter 5.3.1]. Fundamental changes in the medical and pharmaco-economic environment are essential for a successful re-introduction of bacteriophage therapy into modern medicine. Bacteriophage therapy fits well in the new emerging field of Darwinian/evolutionary medicine where the insights of evolution are fully taken into account. Viruses, among which are bacteriophages, were involved in the origin of life itself and play a major role in biological evolution [Villarreal 2005, Forterre 2006, Koonin 2006]. Hopefully, they will play a more important role in the future control of bacterial diseases. We must learn from the errors that contributed to the rise of antibiotic resistance. This vision has to be fostered in collaboration with the competent authorities and responsible political and economic actors, as only a common effort will make bacteriophage therapy a (direly needed) reality. The setting up of credible studies to gather the required data with regard to the modern evidence based efficacy paradigm and the evolutionary consequences of bacteriophage therapy needs to be stimulated. The development of a specific legislative framework with realistic production and documentation requirements that allow a timely supply of safe, tailored natural bacteriophages has to be promoted. Products for bacteriophage therapy deserve their specific (European) regulatory frame. Various pharmaceutical products different from the classical chemical molecules such as vaccines and cell therapy products already have their proper frames [Chapter 4.1.1].

Natural bacteriophages are by definition “products” in the pharmaco-legal jargon. They are natural lifelike biological entities. They proved, although mostly empirically, to have a real value in medical practice in the former Soviet Republics. Bacteriophages are still a therapeutic tool in daily medicine in countries like Georgia and Poland. Bacteriophages could be considered a type of Particular medicinal products, to be defined under Annex 1 Part III of Human Medicinal Products Directive 2001/83/EC [Table 1]. Bacteriophages could be categorized at potentially two classification places. A first

possibility is the creation of a new and specific “Biological medicinal products” categorical ranking place under the name “Bacteriophage medicinal products” at the same rank level as “Plasma-derived medicinal products” and “Vaccines”. The second possibility could be the creation of a dedicated new category of the Part III Particular medicinal products division, also named “Bacteriophage medicinal products”. Both proposed new legal frame options are visualized in Table 1 (*Italic*/**bold**) and concern as “products” only the natural exclusively lytic natural bacteriophages, in contrast to potential useful “genetically engineered” or “bacteriophage derived” products which are not in the scope of this dissertation.

Table 1: Annex 1 Part III of Human Medicinal Products Directive 2001/83/EC in which the bacteriophage therapy concept could be integrated or classified (*Italic*/**bold**).

Part I: Standardized marketing authorisation dossier

Part II: Specific marketing authorisation dossier

- Well-established medicinal use
- Essentially similar medicinal products
- Additional data required in specific situations
- Similar biological medicinal products
- Fixed combination medicinal products
- Documentation for applications in exceptional circumstances
- Mixed marketing authorisation applications

Part III: Particular medicinal products

- Biological medicinal products
  - Plasma-derived medicinal products
  - Vaccines
  - ***Bacteriophage medicinal products***
- Radio-pharmaceuticals and precursors
  - Radio-pharmaceuticals
  - Radio-pharmaceutical precursors for radio-labelling purposes
- Homeopathic medicinal products
- Herbal medicinal products
- Orphan medicinal products
- ***Bacteriophage medicinal products***

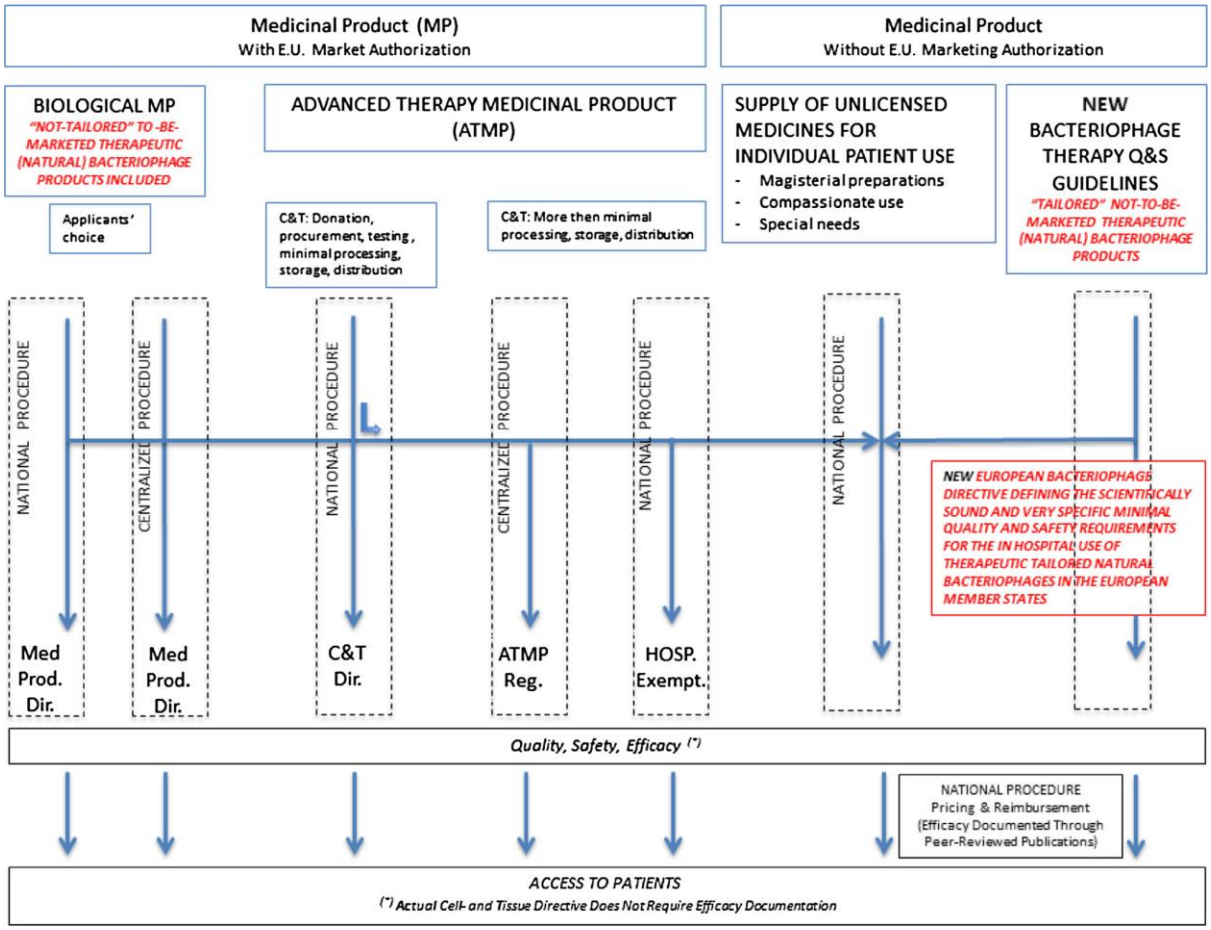
Part IV: Advanced Therapy Medicinal Products

- Gene therapy medicinal products
- Somatic cell therapy medicinal products
- Tissue engineered products
- Combined advanced therapy medicinal products

7.1.2 Options outside the Medicinal Products Directive 2001/83/EC: European (centralized) or national (de-centralized) procedure

An alternative pathway could be the creation of a new and dedicated Bacteriophage Therapy Directive, outside the existing Human Medicinal Products Directive 2001/83/EC [Chapter 5.1.1 Fig. 2].

Chapter 5.1.1 Fig. 2: Proposal for a new European directive for bacteriophage therapy.



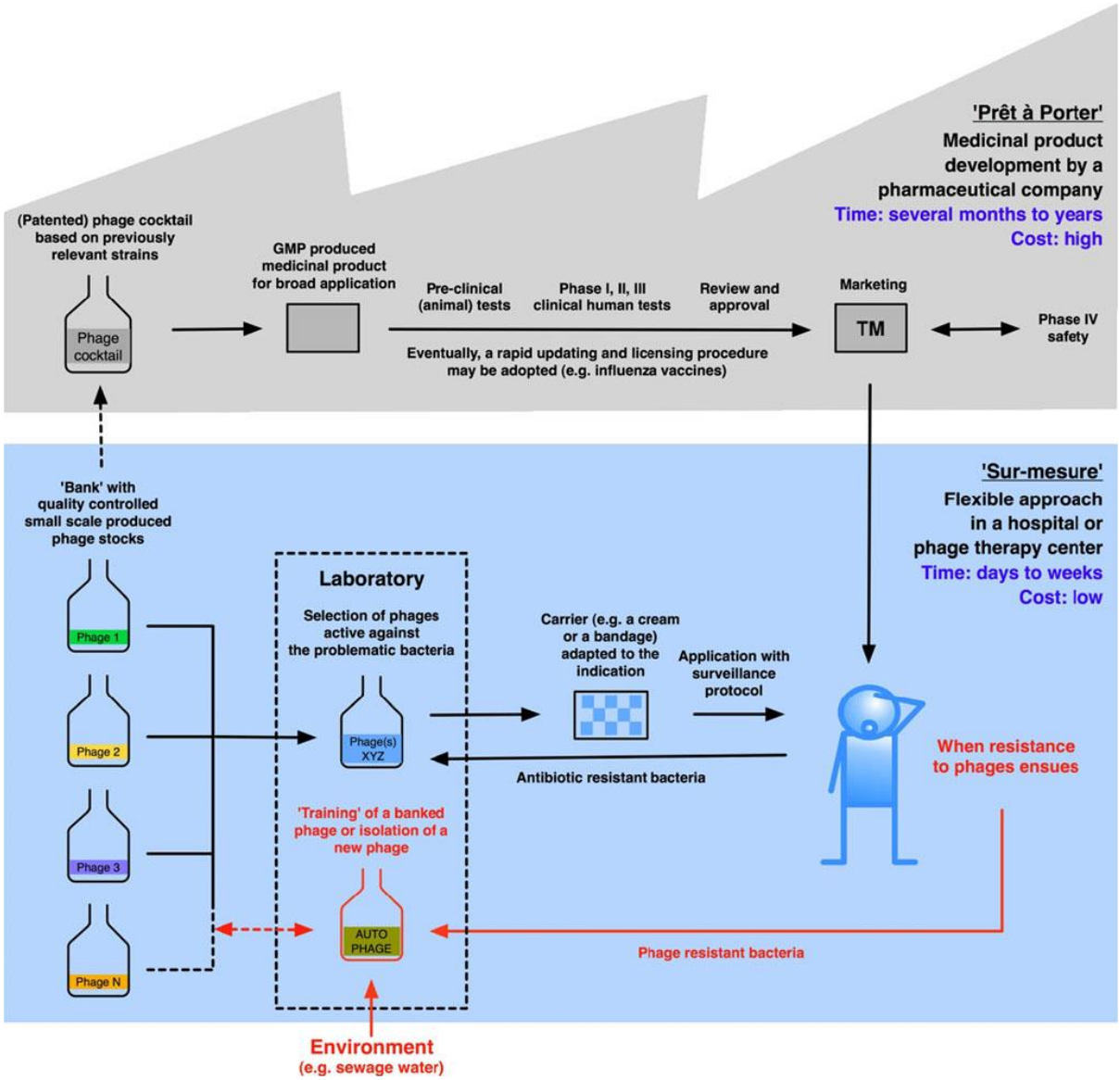
In both pathways followed (inside or outside the Human Medicinal Products Directive 2001/83/EC) the frames tailored to bacteriophage therapy should allow an adapted developmental timeframe. The development of a ‘‘new’’ natural bacteriophage “product” can in practice happen in a matter of days to weeks [Merabishvili 2012], in contrast to the timeframes applicable to the developing of classical chemical antibiotics. This development is in line with the so called Quality by Design (QbD) drug development principle [Chapter 3.1.1 Fig. 1, Chapter 4.1.1], in use for example in the field of therapeutic human cell culture and vaccine production. The new or adapted frameworks should also make the tailored use of bacteriophage therapy in hospitals or recognized bacteriophage therapy centres possible.

Regulatory authorities have the responsibility to survey the application of the existing regulations. Actually a specific regulation is lacking for bacteriophage therapy. Hence there is a need for a legislative action at the political level. We suggest a regulatory framework, inspired by the existing

legislation governing the Advanced Therapy Medicinal Products (ATMPs), which includes a “Hospital-Exemption” (HE) for the hospital-based use of cell and gene-based products and therapies. The European Medicines Agency (EMA) recently classified natural therapeutic bacteriophages as Biological Medicinal Products (BMPs). Actually however, a defined HE does not exist under the Biological Medicinal Products legislative frame [Chapter 4.2.1, Chapter 5.2.1].

An optimized regulatory framework would distinguish between the hospital-based (tailor-made) “Sur-Mesure” use of natural bacteriophages in patients and the industrial production and distribution of uniform “Prêt-a-Porter” bacteriophage products. This would allow treating physicians to fully exploit the co-evolutionary aspects of natural bacteriophages to their patients’ benefit [Chapter 2.3.1, Chapter 3.1.1 Fig. 1, Chapter 3.1.3].

Chapter 3.1.1 Fig. 1: Two bacteriophage therapy concepts: “Prêt-a-Porter” and “Sur-Mesure”



In case the European Union would not actively support, in the near future, the re-introduction of tailored bacteriophage therapy into its Member States, national authorities of these Member States could also regulate bacteriophage therapy at their level. There is an urgent need for bacteriophage therapy centres seen the increasing antibiotic resistances and its negative consequences. If in the actual pharmaco-economic environment the launch of hospital-based (non-profit) bacteriophage therapy centres should be financially impossible, national governments should support those initiatives and eventually help to develop new pharmaco-economic models.

7.2 Complementary actions for re-introducing bacteriophage therapy into the European Union: a bottom-up approach

Since several years there is an increasing amount of actions undertaken in order to bring the therapeutic potentialities of bacteriophage therapy also under the consciousness of a broader public, consisting of the patients, the first line medical community, the politicians as decision makers and society as a whole. Therefore several societies organise information sessions or new associations are specifically created. As well at the national as at the international level, dedicated bacteriophage therapy symposia and workshops are organised. Examples are the “Viruses of Microbes” conference [Switzerland 2014], the “Bacteriophage 2015 & Bacteriophage 2016” conferences [Oxford 2015, London 2016] and the “Phages in Interacation” symposium [Leuven 2015]. Actually, a big change is observed among the attitude of the competent authorities now also organizing meetings about the subject. A situation that was almost unthinkable a few years ago. The European Medicines Agency (EMA) recently organized a workshop concerning the therapeutic use of bacteriophages [EMA 2015] while the Joint Research Centre (JRC) of the European Commission organized a meeting where also bacteriophage therapy was put on the agenda [JRC 2013]. These initiatives are important at the scientific and technical industrial level, as well as for the creation of a positive public opinion and political awareness at the decision making level. Some initiatives led by non-profit organizations like GEEPhage [GEEPhage], P.H.A.G.E. [P.H.A.G.E.] and PHAG ESPOIRS [PHAGESPOIRS] play an active and primary role for change, particularly due to a co-involvement of patient organizations. Another positive evolution is that organizations like the World Alliance Against Antibiotic Resistance include bacteriophages as a potential help in the fight against antibiotic resistance [WAAAR 2015].

7.3 The moral issue

There are moral arguments for taking the re-introduction of bacteriophage therapy into (Western) medicine seriously [Chapter 3.1.2, Chapter 6.1.1]. All stakeholders (pharmaceutical industry, competent authorities, lawmakers, regulators, and politicians) have the moral duty and the shared responsibility towards making bacteriophage therapy urgently available for all patients in need [Chapter 6.1.1]. The pharmaceutical industry has a moral duty to invest in bacteriophage therapy in view of the social responsibility they need to take. Of equally crucial importance is the role of the competent public authorities to create the appropriate legislative framework to stimulate companies to

invest in bacteriophage therapy. Political representatives and lawmakers have an inevitable, logical responsibility to support health care and welfare. The development and production of bacteriophage products in a classical pharmaceutical context (clinical trials, production requirements, marketing authorization procedures...) requires much time. Patients in need have no time. Therefore, lawmakers and regulators need to design appropriate solutions, on a short term, to buffer for the years needed for companies to develop bacteriophage-based medicinal products. Much more urgent and optimal, legislative solutions need to be created to allow hospitals to adopt patient-oriented and tailored bacteriophage therapy in a legal way for treating those patients that are waiting to be cured today. For these patients in need, bacteriophage therapy as it stands today can be labelled as an Ethically Justified Medical Therapy (EJMT) [Kagan 1998, Miller 1996]. This means that, although it has not yet been officially approved, the use of bacteriophage therapy is morally justifiable if and only if the EJMT-related criteria are satisfied (Table 2) [Chapter 6.1.1].

Table 2: Six criteria for a therapy to be labelled as Ethically Justified Medical Therapy.

It is morally permissible to set aside the *prima facie duty* not to impose a risk of harm if and only if	
(i)	there is a just cause;
(ii)	those who want to put aside the duty not to impose a risk of harm have good intentions;
(iii)	there is a reasonable chance that the just cause will be realized;
(iv)	the harm prevented will outweigh the risk of harm imposed;
(v)	the just cause cannot be obtained with at least the same probability of success but without imposing a risk of harm;
(vi)	those who decide on putting aside the duty not to impose a risk of harm constitute a legitimate authority.

7.4 Bacteriophage therapy concepts

Ninety years of bacteriophage therapy have shown that after a while bacteriophage preparations become less effective and need to be updated [Chanishvili 2012]. The ineffective bacteriophages can either be “trained,” a term used in the Georgian Eliava Institute of Bacteriophage Microbiology and Virology (EIBMV), to indicate the selection of bacteriophage mutants more active against the bacteriophage-resistant bacteria, or replaced by new active bacteriophages. New bacteriophages are generally selected from the environment (e.g. hospital sewage water), but in some cases they can be isolated from clinical samples containing the problematic bacterium. In bacteriophage therapy centres in Georgia and Poland, therapeutic bacteriophage banks containing many different bacteriophages are kept and regularly updated. Sometimes custom bacteriophage preparations are developed for a patient’s infection, a procedure that usually takes a few days to weeks. This “Sur-Mesure” approach is not compatible with the current (medicinal product) licensing processes [Chapter 3.1.1].

EMA recently classified natural therapeutic bacteriophages under the (classical) Medicinal Product Legislation. Also, in the US, the amount of research and testing required by the FDA is seriously hampering the resurgence of bacteriophage therapy. Notwithstanding these regulatory hurdles and the empirical evidence suggesting that stable and widely distributed bacteriophage preparations (“Prêt-à-Porter”) will only be of (time-)limited use, a few companies have picked up the gauntlet and are moving along the elaborate and expensive licensing pathways. If nothing else, these efforts will put bacteriophage therapy back on the map in the “Western World” and, once commonly accepted,

EMA and FDA might revise their rules the way they did for influenza vaccines which also require a rapid updating and licensing procedure [Wood 2003].

However, are pharmaceutical companies willing to commit to rapidly and regularly adapting their bacteriophage preparations to very specific or newly emerging demands? E.g. for a hospital unit confronted with a multi-drug resistant bacterial strain that causes untreatable infections in only one or two of its patients? For all these reasons, as well the “Prêt-à-Porter” or the “Sur-Mesure” approaches should be developed. Operational, they even could be complementary [Chapter 3.1.2]

The specificity of bacteriophages, resistance and intellectual property (IP) issues may hamstring pharmaceutical companies in the worldwide marketing of tailored bacteriophage preparations. Indeed, the lack of strong IP protection is a discouraging factor for pharmaceutical companies [Chapter 2.1.1]. The principle of bacteriophage therapy has been common knowledge since the 1920s and many aspects might thus be un-patentable. In addition, there are indications that in the future bacteriophages, which are natural entities composed of genetic material and proteins, will only be patentable if they have been engineered into something distinctly different in character. Engineered bacteriophages could be patented but, considering the current concerns about potential risks for public health and the environment which may arise from genetic engineering in genetically modified organisms (GMOs), they are not likely to be given licensing approval as a medicinal product in the near future.

The long and expensive regulatory pathways form insurmountable obstacles for bonafide non-profit bacteriophage therapy centres or hospitals which opt for a “Sur-Mesure” concept, as well as for institutions that would like to use bacteriophages for commercially unattractive applications, in e.g. emerging countries. Also this “Sur-Mesure” conceptual approach should adhere to relevant standards of safety, quality and efficacy [Chapter 2.2.1, Chapter 5.2.1].

Bacteriophage therapy (in general) has great potential but, as with antibiotic treatment, there are likely to be important evolutionary consequences [Levin 2004] if bacteriophage therapy is implemented widely and without sufficient oversight. Some aspects of the bacteriophage/bacterium evolution ecology (e.g. emergence of resistance) should be analysed in the light of bacteriophage therapy. Real-time experimental evolution studies could help determine these evolutionary consequences and generate the analytical knowledge in support of the empirical knowledge and clinical experience that was accumulated in the “Eastern World”. More importantly, they will hopefully enable the creation of a rational bacteriophage therapy concept, avoiding the historical mistakes that occurred in the course of antibiotic therapy development and which lead to the current massive and widespread occurrence of antibiotic resistance in the patient population as well as in the natural environment.

7.5 Re-thinking the European Medicinal Products Directive 2001/83/EC and evaluating its scope

European Human Medicinal Product legislation needs to be reworked in relation to the re-introduction of bacteriophage therapy into the European Union. The creation of a HE (comparable to the HE already defined for the ATMPs) could make tailored in-hospital use of bacteriophages possible. Products falling under the HE definition will no longer be covered by the scope of the European Medicinal Product Directive 2001/83/EC (Art. 3.7)[1] [EMPD 2001, Cuende2014, Belgian SHC Advice 9218].

It could be contended that the tailored production and the in-hospital use of natural therapeutic bacteriophages do not constitute a market placement of that product. This places these products and their use again outside the scope of European Medicinal Product Directive 2001/83/EC (Art. 2.1)[2] [Chapter 4.2.1, Bredin 2012]. Both arguments motivate for the creation of a totally new and specific European bacteriophage therapy directive.

Last but not least the European Medicinal Product Directive 2001/83/EC itself foresees that Member States could declare the European Medicinal Product Directive not being applicable in relation to their special national needs (Art.5.1)[3]. As such, e.g. in Belgium, it is possible that a recognized physician orders the production of a specific bacteriophage therapeutic product, based on his own specifications, for use on his own patients, under his sole responsibility [Belgian SHC Advice 9218].

7.6 Final reflexions

We have to conclude that a continuous talk and interaction with all the actors in the field is of prime necessity. Doing nothing to address the growing bacterial resistance to antibiotics is not an option considering that 25.000 European citizens die annually from untreatable bacterial infections [Chapter 5.1.1, Ackermann 2012]. Europe and its Member States should take their responsibility to financially, technically and legally support the re-introduction of bacteriophage therapies into the European Union. A collective action at the European political level can be an option for securing a vote of the European Parliament to adapt the actual European Medicinal Product legislative frame in view of these statements.

---

[1] Art. 3.7 >This Directive shall not apply to: Any advanced therapy medicinal product, as defined in Regulation (EC) No 1394/2007, which is prepared on a non- routine basis according to specific quality standards, and used within the same Member State in a hospital under the exclusive professional responsibility of a medical practitioner, in order to comply with an individual medical prescription for a custom-made product for an individual patient.

[2] Art. 2.1 > Scope: This Directive shall apply to medicinal products for human use intended to be placed on the market in Member States and either prepared industrially or manufactured by a method involving an industrial process.

[3] Art. 5.1 > A Member State may, in accordance with legislation in force and to fulfil special needs, exclude from the provisions of this Directive medicinal products supplied in response to a bona fide unsolicited order, formulated in accordance with the specifications of an authorised health-care professional and for use by an individual patient under his direct personal responsibility.

7.7 Disclosure

Gilbert VERBEKEN (author of this dissertation and (co-)author of the scientific publications included in this dissertation) complies with the recommendation of the International Committee of Medical Journal Editors [ICMJE] concerning scientific authorship.
The International Committee of Medical Journal Editors [ICMJE] recommends that authorship be based on the following 4 criteria:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he or she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

7.8 Remarque

During all thesis-related discussions and contacts, I have never encountered real opposition to the idea of a smooth and qualitative re-introduction of bacteriophage therapy into the European Union. On the other hand, what I regularly came across, were “believers” and “non-believers” when discussing the potential of natural lytic bacteriophages to solve the (*in-vivo*) problem of antimicrobial resistance to antibiotics. Therefore and again, “state-of-the-art” clinical trials (control-groups included) are a pre-requisite to go fast-forward in this promising and lifesaving therapeutic field.

7.9 References

Ackermann HW (2012) Who went into phage research? Bacteriophage 2:55-59

Belgian SHC Advice 9218 (2015) Advice on the Hospital Exemption as defined for the Advanced Therapy Medicinal Products (ATMPs)

Bredin (2012) Avis hôpital militaire Reine Astrid - Keratinocytes (in French), Bredin-Prat lawyers office

Chanishvili N (2012) A literature review of the practical application of bacteriophage research. ISBN-13:978-1621008514; ISBN-10:1621008517; 1st Edition (Book); Nova Science Publishers

Chapter 2.1.1; De Vos D, Verbeken G, Ceulemans C, Huys I, Pirnay JP (2014) Reintroducing phage therapy in modern medicine: the regulatory and intellectual property hurdles. In: Phage Therapy Current Research and Applications (Chapter 12); Caister Academic Press; ISBN: 978-1-908230-40-9

Chapter 2.1.2; Verbeken G, De Vos D, Vaneechoutte M, Merabishvili M, Zizi M, Pirnay JP (2007) European regulatory conundrum of phage therapy. Future Microbiol 2(5):485-491

Chapter 2.2.1; Merabishvili M, Pirnay JP, Verbeken G, Chanishvili N, Tediashvili M, Lashkhi N, Glonti T, Krylov V, Mast J, Van Parys L, Lavigne R, Volckaert G, Mattheus W, Verween G, De Corte P, Rose T, Jennes S, Zizi M, De Vos D, Vaneechoutte M (2009) Quality-controlled small-scale production of a well-defined bacteriophage cocktail for use in human clinical trials. PLoS ONE 4(3):e494 DOI: 10.1371/journal.pone.0004944.

Chapter 2.3.1; Rose T, Verbeken G, De Vos D, Merabishvili M, Vaneechoutte M, Lavigne R, Jennes S, Zizi M, Pirnay JP (2014) Experimental phage therapy of burn wound infection: difficult first steps. Int J Burn Trauma 4(2):66-73

Chapter 2.3.2; De Vos D, Verbeken G, Rose T, Jennes S, Pirnay JP (2012) Bacteriophages for the treatment of severe infections: a “new” option for the future. EWMA Journal 12(2):23-28

Chapter 3.1.1; Pirnay JP, De Vos D, Verbeken G, Merabishvili M, Chanishvili N, Vaneechoutte M, Zizi M, Laire G, Lavigne R, Huys I, Van den Mooter G, Buckling A, Debarbieux L, Pouillot F, Azeredo J, Kutter E, Dublanchet A, Górski A, Adamia R (2011) The phage therapy paradigm: prêt-à-porter or sur-mesure? Pharm Res 28(4):934-937 DOI:10.1007/s11095-010-0313-5

Chapter 3.1.2; Pirnay JP, Verbeken G, Rose T, Jennes S, Zizi M, Huys I, Lavigne R, Merabishvili M, Vaneechoutte M, Buckling A, De Vos D (2012) Introducing yesterday’s therapy in tomorrow’s medicine. Future Virol.7 (4), 379-390 │10.2217/FVL.12.24©2012 Future Medicine Ltd

Chapter 3.1.3; Huys I, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G (2013a) Paving a regulatory pathway for phage therapy: Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. EMBO reports 14(11):951-954 DOI 10.1038/embor.2013.163

Chapter 4.1.1; Verbeken G, Pirnay JP, De Vos D, Jennes S, Zizi M, Lavigne R, Casteels M, Huys I (2012) Optimizing the European regulatory framework for sustainable bacteriophage therapy in human medicine. Arch Immunol Ther Exp DOI:10.1007/s00005-012-0175-0

Chapter 4.2.1; Verbeken G, De Vos D, De Coninck A, Roseeuw, Kets E, Vanderkelen A, Draye JP, Rose T, Jennes S, Huys I, Ceulemans C, Pirnay JP (2015) Bacteriophage therapy, fast-forward to the past: lessons identified from the advanced therapy regulation. Burns (Submitted April 2015)

Chapter 5.1.1; Verbeken G, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Huys I (2014a) Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp DOI:10.1007/s00005-014-0269-y

Chapter 5.2.1; Pirnay JP, Blasdel BG, Bretaudeau L, Buckling A, Chanishvili N, Clark JR, Corte-Real S, Debarbieux L, Dublanchet A, De Vos D, Gabard J, Garcia M, Goderdzishvili M, Górski A, Hardcastle J, Huys I, Kutter E, Lavigne R, Merabishvili M, Olchawa E, Parikka KJ, Patey O, Pouilot F, Resch G, Rohde C, Scheres J, Skurnik M, Vaneechoutte M, Van Parys L, Verbeken G, Zizi M, Van den Eede G (2015) Quality and safety requirements for sustainable phage therapy products. Pharm Res DOI:10.1007/s11095-014-1617-7

Chapter 5.3.1; Huys I, Vaneechoutte M, Verbeken G, Debarbieux L (2013b)
Key issues in phage therapy: A report of a dedicated workshop at the viruses of microbes II meeting. ResMicrobiol 164(7):806-10 DOI:10.1016/j.resmic.2013.03.020

Chapter 6.1.1; Verbeken G, Huys I, Pirnay JP, Jennes S, Chanishvili N, Scheres J, Górski A, De Vos D, Ceulemans C (2014b) Taking bacteriophage therapy seriously: a moral argument. Biomed Res Int 621316 DOI:10.1155/2014/621316

Cuende N, Boniface C, Bravery C, Forte M, Giordano R, Hildebrandt M, Izeta A, Dominici M (2014) on behalf of the legal and regulatory affairs committee-Europe, international society cellular therapy. The puzzling situation of hospital exemption for advanced therapy medicinal products in Europe and stakeholders' concerns. Cytotherapy 16:1597-1600

EMA (2015) Workshop on the therapeutic use of bacteriophages, European Medicines Agency, June 12th 2015, London, UK
http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/events/2015/05/event_detail_001155.jsp&mid=WC0b01ac058004d5c3

EMPD 2001. European Directive 2001/83/EC of the European parliament and of the council on the Community code relating to medicinal products for human use of 6 November 2001; 2001L0083 - EN - 16.11.2012 - 011.001 - 1 (Compiled; 2014)
http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and__procedural_guideline/2009/10/WC500004481.pdf

Forterre P (2006) The origin of viruses and their possible roles in major evolutionary transitions. Virus Res 117(1):5-16

GEEPhage http://www.geephage.org/joomla/

ICMJE: http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html

JRC (2013) Scientific support for public health: existing actions, new challenges and European added value. Joint Research Centre, European Commission, 14-15 Nov 2013, Brussels, Belgium

Kagan S (1998) Normative ethics. Westview Press, Colorado, USA

Koonin EV, Senkevich TG, Dolja VV (2006) The ancient Virus World and evolution of cells. Biol Direct 1:29

LevinBR, Bull JJ (2004) Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol 2:166-173

Leuven (2015) Conference, Phages in Interaction, Sept. 29th 2015, KU Leuven, Leuven, Belgium

London (2016) Conference, Bacteriophage 2016, 19th - 21st January 2016, London, UK
http://lifescienceevents.com/bacteriophage-2016-19th-21st-january-2016/

Merabishvili M, De Vos D, Verbeken G, Kropinski A, Vandenheuvel D, Lavigne R, Wattiau P, Mast J, Ragimbeau C, Mossong J, Scheres J, Chanishvili N, Vaneechoutte M, Pirnay JP (2012) Selection and characterization of a candidate therapeutic bacteriophage that lyses the *Escherichia coli* O104:H4 strain from the 2011 outbreak in Germany. PLoS ONE 7(12):e52709

Miller RB (1996) Casuistry and modern ethics: a poetics of practical reasoning. University of Chicago Press, London, UK

Oxford (2015) Conference, Bacteriophage 2015, St Hilda's College, Oxford, UK http://10times.com/oxford-bacteriophage-conference

P.H.A.G.E. http://www.p-h-a-g-e.org/

PHAG ESPOIRS http://phagespoirs.unblog.fr/

Switzerland (2014) Conference, Viruses of Microbes Conference.17-18 July 2015, Zurich, Switzerland

Villarreal LP (2005) Viruses and the evolution of life. ASM Press. DOI:10.1128/9781555817626

WAAAR (2015) http://www.globalhealthdynamics.co.uk/wp-content/uploads/2015/05/20_waaar.pdf

Wood JM, Levandowski RA (2003) The influenza vaccine licensing process. Vaccine 21:1786-1788

8 Summary

8. Summary

The worldwide emergence of antibiotic resistant bacteria and constraints to investment in potential solutions may eventually lead to a return to the pre-antibiotic era. As industries' antibiotic pipeline is virtually dry and infectious diseases are steadily on the increase, the use of bacteriophages (bacteriophages are bacterio-specific viruses) to kill bacteria can be considered as a valuable option. Bacteriophages (meaning "bacteria-eaters") are the bacteria's natural enemies. In combination with or as substitute for antibiotics, bacteriophage therapy could be a therapeutic option in the eradication or control of bacterial colonization/infections. Bacteriophages can be considered as self-amplifying therapeutic products. By setting up a screening system for the circulating noxious bacteria and their respective bacteriophages it will be possible to obtain the right bacteriophage against any emerging pathogen.

Bacteriophages were discovered independently during World War I by the French-Canadian biologist Felix d'Herelle and by the English microbiologist Frederick Twort. d'Herelle developed a commercial laboratory in Paris that produced and distributed bacteriophage preparations against various bacterial infections. In the 1930s, therapeutic bacteriophages were also marketed in the United States by major pharmaceutical companies. The advent of antibiotics relegated bacteriophage therapy to complete obscurity in most of the Western world.

This PhD project aims at contributing to the creation of a dedicated European regulatory framework that makes the smooth re-introduction of bacteriophage therapy in the European Union possible. The research hypothesis is that final reflections and proposals, specifically designed in relation to bacteriophage therapy, will offer new and usable insights to all stakeholders involved. This research (11 studies) resulted in 14 international scientific publications that form the core of this thesis manuscript.

Chapter 1 of this manuscript is written as a general introduction and describes the natural bacteriophage, the problem of bacterial resistance development to antibiotics, the potential of natural bacteriophages in tackling this problem, the history of bacteriophage therapy and the actual European medicinal product regulatory setting relevant to the therapeutic use of bacteriophages. This chapter also contains an overview of the PhD project, including the research objectives.

Chapter 2 investigates the actual European regulatory and intellectual property hurdles relevant to the re-introduction of bacteriophage therapy into the European Union. This chapter also describes what a small-scale production of a qualitative bacteriophage cocktail, meant for use in e.g. a clinical trial, could look like. These investigations (and their results) intrigued regulators, politicians and medical ethical committees. The regulatory discussion on the subject of "bacteriophage therapy" was opened.

Chapter 3 explains what an optimal regulatory pathway, tailored to bacteriophage therapy, could look like. It explains why bacteriophage therapy is probably best served by a tailor-made and sustainable bacteriophage therapy concept.

Chapter 4 compares the bacteriophage therapy concept (explained in Chapter 3) with other medicinal products and assesses the compatibility of this bacteriophage therapy concept with current attitudes of national and European regulatory agencies towards bacteriophage products. Chapter 4 also analyses if the European Advanced Therapy Medicinal Products (ATMPs) Regulation, an example of an existing adapted medicinal product legislative framework, could be tailored to also cater for flexible bacteriophage therapy concepts.

Chapter 5 proposes a dedicated European regulatory frame for bacteriophage therapy, including quality and safety requirements for sustainable bacteriophage therapy products. These requirements are consensus-requirements defined by 33 bacteriophage experts from 11 different countries. The proposed regulatory framework was validated during an international workshop that took place at the Belgian Royal Military Academy (Viruses of Microbes II, Brussels, Belgium).

Chapter 6 investigates all stakeholders' moral responsibility in relation to the large-scale re-introduction of bacteriophage therapy into the European Union. Moral principles are investigated and moral arguments are formulated in an effort to motivate all stakeholders to take bacteriophage therapy seriously. Although the efficacy of bacteriophage therapy has not been proven according to the actual standards of the European Union, bacteriophage therapy can be considered to be an Ethically Justified Medical Therapy (EJMT) within the European Union.

Chapter 7 is written in the format of a concluding discussion summarizing legislative proposals that could work for the conceptual re-introduction of natural bacteriophage therapy into the European Union.

9 Samenvatting

9. Samenvatting

De wereldwijde opkomst van antibiotica resistente bacteriën en het gebrek aan investeringen teneinde dit probleem grondig aan te pakken zou kunnen leiden tot een terugkeer naar het pre-antibiotica tijdperk. Aangezien de industriële antibiotica pijplijn virtueel droog is en de infectieuze ziekten aan een permanente opmars bezig zijn, zou het gebruik van bacteriofagen (bacteriofagen zijn bacterie-specifieke virussen) als een waardevolle optie kunnen worden beschouwd. Bacteriofagen (wat “bacteriën-eters” betekent) zijn de natuurlijke vijanden van bacteriën. In combinatie met of als vervanging van antibiotica zou bacteriofaag therapie een therapeutische optie kunnen zijn voor wat het uitroeien of controleren van bacteriële kolonisaties/infecties betreft. Bacteriofagen kunnen gezien worden als zelf-vermenigvuldigende therapeutische producten. Door een screening systeem op te zetten voor de circulerende ziekteverwekkende bacteriën en hun overeenkomstige bacteriofagen wordt het mogelijk de juiste bacteriofaag tegenover om het even welke opkomende ziekteverwekkende bacterie te vinden.

Bacteriofagen werden gedurende Wereld Oorlog I, onafhankelijk van elkaar, ontdekt door de Frans-Canadese bioloog Felix d’Herelle en de Engelse microbioloog Frederick Twort. d’Herelle ontwikkelde in Parijs een commercieel laboratorium dat bacteriofaagpreparaten tegen verschillende bacteriële infecties produceerde en distribueerde. In de jaren ‘30 werden therapeutische bacteriofagen ook in de Verenigde Staten gecommercialiseerd en dat door grote farmaceutische bedrijven. De opkomst van antibiotica verdrong bacteriofaag therapie in het grootste deel van de Westerse wereld.

Dit PhD project heeft tot doel bij te dragen tot de vormgeving van een specifiek Europees regelgevend kader dat de vlotte (re-) introductie van bacteriofaag therapie in de Europese Unie moet mogelijk maken. De onderzoekshypothese is dat finale reflecties en voorstellen, specifiek ontwikkeld in relatie tot bacteriofaag therapie, nieuwe en bruikbare inzichten kunnen bieden aan alle betrokken stakeholders. Het gevoerde onderzoek (11 studies) resulteerde in 14 internationale wetenschappelijke publicaties. Zij maken samen de kern uit van dit thesis-manuscript.

Hoofdstuk 1 van dit manuscript is geschreven als een algemene inleiding en beschrijft de natuurlijke bacteriofaag, het probleem van de ontwikkeling van bacteriële resistentie aan antibiotica, het potentieel van natuurlijke bacteriofagen ter oplossing van dit probleem, de geschiedenis van bacteriofaag therapie en het actuele Europese medicinale producten regelgevende kader relevant voor het therapeutisch gebruik van bacteriofagen. Dit hoofdstuk bevat tevens een overzicht van het PhD project, de onderzoek objectieven inbegrepen.

Hoofdstuk 2 onderzoekt de actuele Europese regelgevende en intellectuele eigendom obstakels relevant voor wat de (re-) introductie van bacteriofaag therapie in de Europese Unie betreft. Dit hoofdstuk beschrijft eveneens hoe een kleinschalige en kwalitatieve productie van een bacteriofaagcocktail, bedoeld voor gebruik in bv een klinisch studie, er uit zou kunnen zien. Deze

onderzoeken (en de resultaten er van) intrigeerden regulators, politici en medisch ethische comités. De regelgevende discussie m.b.t. “bacteriofaag therapie” werd hierbij geopend.

Hoofdstuk 3 beschrijft een mogelijk optimaal regelgevend kader, specifiek voor bacteriofaag therapie. Het legt uit waarom bacteriofaag therapie waarschijnlijk beter af is met een op maat gemaakt en duurzaam bacteriofaag therapie concept.

Hoofdstuk 4 vergelijkt het bacteriofaag therapie concept (uitgelegd in Hoofdstuk 3) met andere medicinale producten en beoordeelt de verenigbaarheid van dit bacteriofaag therapie concept met actuele houdingen van nationale en Europese regelgevende autoriteiten tegenover bacteriofaag producten. Hoofdstuk 4 analyseert eveneens of de Europese regelgeving m.b.t. “geavanceerde therapie” medicinale producten (ATMPs), een voorbeeld van een bestaand aangepast regulerend kader voor medicinale producten, zou kunnen worden aangepast zodat het ook toepasbaar wordt op flexibele bacteriofaag therapie concepten.

Hoofdstuk 5 stelt een specifiek “bacteriofaag therapie” Europees regelgevend kader voor, inclusief kwaliteit- en veiligheidsvereisten voor duurzame bacteriofaag therapie producten. Deze vereisten zijn consensus-vereisten gedefinieerd door 33 bacteriofaag-experten uit 11 verschillende landen. Het voorgestelde regelgevende kader werd gevalideerd gedurende een internationale workshop die plaats vond aan de Belgische Koninklijke Militaire School (Viruses of Microbes II, Brussels, Belgium).

Hoofdstuk 6 onderzoekt de morele verantwoordelijkheid van alle stakeholders in relatie tot de grootschalige (re-) introductie van bacteriofaag therapie in de Europese Unie. Morele principes worden onderzocht en morele argumenten worden geformuleerd met als doel alle stakeholders te motiveren bacteriofaag therapie ernstig te nemen. Ondanks het feit dat de werkzaamheid van bacteriofaag therapie nog niet bewezen is in overeenstemming met de standaarden actueel gehanteerd door de Europese Unie, kan bacteriofaag therapie toch gezien worden als een Ethisch Gerechtvaardigde Medische Therapie (EJMT) binnen de Europese Unie.

Hoofdstuk 7 is geschreven in het formaat van een afsluitende discussie die een samenvatting geeft van de regelgevende voorstellen die geschikt kunnen zijn voor de conceptuele (re-) introductie van natuurlijke bacteriofaag therapie in de Europese Unie.

10 Curriculum vitae

10 Curriculum vitae

10.1 Professional career

2006 – Present:

- Senior Scientist (Biologist) & QA/QC/RA Manager at Queen Astrid Military Hospital[1]
- Scientific Co-Operator Free University Brussels (Faculty of Medicine)

2007 – Present:

- Expert Advisor on Human Cell- and Tissue Banking (Belgian Superior Health Council)

2008 – Present:

- Expert Advisor on Human Cell- and Tissue Banking (EuroGTP) (Eur. Commission, DG Sanco)

2009 – Present:

- Expert Scientific Advisor Public Health and Risk Assessment (Eur. Commission, DG Sanco)

2010 – Present:

- Expert Evaluator of Research Proposals (Ethical Review) (Eur. Commission, DG Research)

2010 – Present:

- Scientific Co-Operator KU Leuven (Faculty of Pharmaceutical Sciences)

2010 – Present:

- PhD Candidate at KU Leuven and Royal Military Academy (Co-Doctorate)

2011 – Present:

- Expert Advisor NATO Research Task Group Human Factors and Medicine (RTG-HFM 194)

2013 – Present:

- Scientific Co-Operator at Royal Military Academy

2001 – 2005:

- Project Manager Human Keratinocyte Cultures at XCELLentis NV
- Project Manager Human Keratinocyte Cultures at Queen Astrid Military Hospital

1989 – 2001:[2]

- Project Manager Wound Healing at Innogenetics NV
- Project Manager Human Keratinocyte- and Skin Banking at Queen Astrid Military Hospital

1988 – 1989:

- Project Manager Human Keratinocyte- and Skin Banking at Queen Astrid Military Hospital

[1] Queen Astrid Military Hospital, Burn Wound Centre, Laboratory for Molecular and Cellular Biology (LabMCT), Human Cell- and Tissue Banks, Neder-Over-Heembeek, Belgium

[2] 1996 – 1997: Interruption of Career > Travel Around the World (Europe, Pacific, Asia, Africa, Latin America)

10.2 Articles in international scientific journals

10.2.1 Peer-reviewed

Boone M., Draye J.P., Verween G., Aita A., Pirnay J.P., Verbeken G., De Vos D., Rose T., Jennes S., Jemec G., Del Marmol V.
RECELLULARISING OF HUMAN ACELLULAR DERMAL MATRICES IMAGED BY HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY
Exp Dermatol 2015 May;24(5):349-54. doi: 10.1111/exd.12662

Pirnay J.-P., Blasdel B., Bretaudeau L, Buckling A., Chanishvili N, Clark J., Corte-Real S., Debarbieux L., Dublanchet A., De Vos D., Gabard G., Garcia M., Goderdzishvili M., Górski A., Hardcastle J., Huys H., Kutter E., Lavigne R., Merabishvili M., Olchawa E., Parikka K., Patey P., Pouilot F., Resch G., Rohde C., Vaneechoutte M., Verbeken G., Zizi M., Van den Eede G.
QUALITY AND SAFETY REQUIREMENTS FOR SUSTAINABLE PHAGE THERAPY PRODUCTS
Pharm Res 2015 Jan 14 [Epub ahead of print] / PMID: 25585954

Rose T., Verbeken G., De Vos D., Merabishvili M., Vaneechoutte M., Lavigne R., Jennes S., Zizi M., Pirnay J.-P.
EXPERIMENTAL PHAGE THERAPY OF BURN WOUND INFECTION: DIFFICULT FIRST STEPS.
Int J Burns Trauma. 2014 Oct 26; 4(2):66-73. eCollection 2014

Merabishvili M., Vandenheuvel D., Kropinski A., Mast J., De Vos D., Verbeken G., Noben J-P, Lavigne R., Vaneechoutte M., Pirnay J-P
CHARACTERISATION OF NEWLY ISOLATED LYTIC BACTERIOPHAGES ACTIVE AGAINST ACINETOBACTER BAUMANNII.
PLoS One. 2014 Aug 11; 9(8):e104853. Doi: 10.1371/journal.pone.0104853. eCollection 2014

M. Boone, J.-P. Draye, G. Verween, J.-P. Pirnay, G. Verbeken, D. De Vos, T. Rose, S. Jennes, G. Jemec, V. de Marmol
REAL-TIME THREE-DIMENSIONAL IMAGING OF EPIDERMAL SPLITTING AND REMOVAL BY HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY.
Exp Dermatol. 2014 Jul 21. Doi: 10.1111/exd. 12516

G. Verbeken, I. Huys, J.-P. Pirnay, S. Jennes, N. Chanishvili, J. Scheres, A. Górski, D. De Vos, C. Ceulemans
TAKING BACTERIOPHAGE THERAPY SERIOUSLY, A MORAL ARGUMENT
Biomed Res Int. 2014; 2014:621316. doi: 10.1155/2014/621316. Epub 2014 Apr 29

G. Verbeken, J.-P. Pirnay, R. Lavigne, S. Jennes, D. De Vos, I. Huys
CALL FOR A DEDICATED EUROPEAN LEGAL FRAME FOR BACTERIOPHAGE THERAPY
Arch. Immunol. et Therap. Experim. DOI 10.1007/s00005-014-0269-y (Published online 06 February 2014)

Huys I, Pirnay JP, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G.
PAVING A REGULATORY PATHWAY FOR PHAGE THERAPY. EUROPE SHOULD MUSTER THE RESOURCES TO FINANCIALLY, TECHNICALLY AND LEGALLY SUPPORT THE INTRODUCTION OF PHAGE THERAPY.
2013 Nov; 14(11):951-4. doi: 10.1038/embor.2013.163. Epub 2013 Oct 18.

J.-P. Pirnay, A. Vanderkelen, D. De Vos, J.-P. Draye, T. Rose, C. Ceulemans, N. Ectors, I. Huys, S. Jennes, G. Verbeken
BUSINESS ORIENTED EU HUMAN CELL AND TISSUE PRODUCT LEGISLATION WILL ADVERSELY IMPACT MEMBER STATES' HEALTH CARE SYSTEMS
Cell Tissue Bank. 2013 Sep; DOI 10.1007/s10561-013-9397-6

Huys I, Vaneechoutte M, Verbeken G, Debarbieux L.
KEY ISSUES IN PHAGE THERAPY: A REPORT OF A DEDICATED WORKSHOP AT THE VIRUSES OF MICROBES II MEETING.
Res Microbiol. 2013 Sep; 164(7):806-10. doi: 10.1016/j.resmic.2013.03.020. Epub 2013 Apr 11.

Merabishvili M, Vervaet C, Pirnay JP, De Vos D, Verbeken G, Mast J, Chanishvili N, Vaneechoutte M.
STABILITY OF STAPHYLOCOCCUS AUREUS PHAGE ISP AFTER FREEZE-DRYING (LYOPHILIZTION).
PLoS One. 2013 Jul 2; 8(7):e68797. doi: 10.1371/journal.pone.0068797. Print 2013.

J. Klykens, J.-P. Pirnay, G. Verbeken, O. Giet, E. Baudoux, R. Jashari, A. Vanderkelen, N. Ectors
CLEAN ROOMS AND TISSUE BANKING: HOW HAPPY I COULD BE WITH EITHER GMP OR GTP?
Cell Tissue Bank. 2013; Jan 04 │DOI 10.1007/s10561-012-9355-8

M. Merabishvili, D. De Vos, G. Verbeken, A.M. Kropinski, D. Vandenheuvel, R. Lavigne, P. Wattiau, J. Mast, C. Ragimbeau, J. Mossong, J. Scheres, N. Chanishvii, M. Vaneechoutte, J.P.-Pirnay
SELECTION AND CHARACTERIZATION OF A CANDIDATE THERAPEUTIC BACTERIOPHAGE THAT LYSES THE ESCHEICHIA COLI O104:H4 STRAIN FROM THE 2011 OUTBREAK IN GERMANY
PLOS ONE 2012; Dec, Vol.7, Issue 12 │e52709

J.-P. Pirnay, A. Vanderkelen, N. Ectors, C. Delloye, D. Dufrane, E. Baudoux, M. Van Brussel, M.P. Casaer, D. De Vos, J.-P. Draye, T. Rose, S. Jennes, P. Neirinckx, G. Laire, M. Zizi, G. Verbeken
BEWARE OF THE COMMERCIALIZATION OF HUMAN CELLS AND TISSUES: SITUATION IN THE EUROPEAN UNION
Cell Tissue Bank. 2012; Jun 21 │DOI 10.1007/s10561-012-9323-3

G. Verbeken, J.-P. Pirnay, D. De Vos, S. Jennes, M. Zizi, R. Lavigne, M. Casteels, I. Huys
OPTIMIZING THE EUROPEAN REGULATORY FRAME FOR SUSTAINABLE BACTERIOPHAGE THERAPY IN HUMAN MEDICINE
Arch. Immunol. et Therap. Experim. 2012; Apr 17 │DOI 10.1007/s00005-012-0175-0

J.-P. Pirnay, G. Verbeken, T. Rose, S. Jennes, M. Zizi, I. Huys, R. Lavigne, M. Merabishvili, M. Vaneechoutte, A. Buckling[1], D. De Vos
INTRODUCING YESTERDAY'S PHAGE THERAPY IN TOMORROW'S MEDICINE
Future Virol. 2012; 7 (4), 379-390 │10.2217/FVL.12.24©2012 Future Medicine Ltd

S. Simoens, G. Verbeken, I. Huys
BIOSIMILARS AND MARKET ACCESS: A QUESTION OF COMPARABILITY AND COSTS?
Target Oncol. 2012; Jan 17 │DOI 10.1007/s11523-011-0192-7

Pirnay JP, Verween G, Pascual B, Verbeken G, De Corte P, Rose T, Jennes S, Vanderkelen A, Marichal M, Heuninckx W, De Vos D.
EVALUATION OF A MICROBIOLOGICAL SCREENING AND ACCEPTANCE PROCEDURE FOR CRYOPRESERVED SKIN ALLOGRAFTS BASED ON 14 DAY CULTURES
Cell Tissue Bank. 2011; Apr 21. [Epub ahead of print] │PMID: 21505962

Peter De Corte, Gunther Verween, Gilbert Verbeken, Thomas Rose, Serge Jennes, Arlette De Coninck, Diane Roseeuw, Alain Vanderkelen, Eric Kets, Jean-Paul Pirnay
FEEDER LAYER- AND ANIMAL PRODUCT-FREE CULTURE OF NEONATAL FORESKIN KERATINOCYTES: IMPROVED PERFORMANCE, USABILITY, QUALITY AND SAFETY
Cell Tissue Bank. 2011; Mar 11. [Epub ahead of print] │PMID: 21394485

Gilbert Verbeken, Gunther Verween, Daniel De Vos, Bruno Pascual, Peter De Corte, Cornelia D Richters, Arlette De Coninck, Diane Roseeuw, Nadine Ectors, Thomas Rose, Serge Jennes, Jean-Paul Pirnay
GLYCEROL TREATMENT AS RECOVERY PROCEDURE FOR CRYOPRESEVED HUMAN SKIN ALLOGRAFTS POSITIVE FOR BACTERIA AND FUNGI
Cell Tissue Bank. 2011; Mar 01. [Epub ahead of print] │PMID: 21360142

Truus Roelandt, Carol Heughebaert, Gunther Verween, Christina Giddelo, Gilbert Verbeken, Jean-Paul Pirnay, Daniel Devos, Debra Crumrine, Diane Roseeuw, Peter M. Elias, Jean-Pierre Hachem
ACTIN DYNAMICS REGULATE IMMEDIATE PAR-2-DEPENDENT RESPONSES TO ACUTE EPIDERMAL PERMEABILITY BARRIER ABROGATION
J. Dermatol Sci 2011 Feb;61(2):101-9│PMID: 21185158│ Epub 07DEC2010

Jean-Paul Pirnay, Daniel De Vos, Gilbert Verbeken, Maia Merabishvili, Nina Chanishvili, Mario Vaneechoutte, Martin Zizi, Geert Laire, Rob Lavigne, Isabelle Huys, Guy Van den Mooter, Angus Buckling, Laurent Debarbieux, Flavie Pouillot, Joana Azeredo, Elizabeth Kutter, Alain Dublanchet, Andrzej Górski, Revaz Adamia
THE PHAGE THERAPY PARADIGM: « *PRÊT À PORTER* » OR « *SUR-MESURE* »?
Pharm Res. 2011 Apr; 28(4):934-7│PMID: 21063753 │ Epub 2010 Nov 10

G. Verbeken, D. Schoeters, G. Verween, P. De Corte, B. Pascual, K. Geukens, A. De Coninck, D. Roseeuw, T. Rose, S. Jennes, Jean-Paul Pirnay
POTENTIAL RELEASE OF ALUMINUM AND OTHER METALS BY FOOD GRADE ALUMINUM FOILS USED FOR SKIN ALLOGRAFT CRYO PRESERVATION
Cell Tissue Bank. │Published 2010 Feb 10 [Epub ahead of print] │PMID: 20146011

Merabishvili M., Pirnay JP, Verbeken G., Chanishvili N., Tediashvili M., Lashkhi N., Glonti T., Krylov V., Mast J., Van Parys L., Lavigne R., Volckaert G., Mattheus W., Verween G., De Corte P., Rose T., Jennes S., Zizi M., De Vos D., Vaneechoutte M.
QUALITY-CONTROLLED SMALL-SCALE PRODUCTION OF A WELL-DEFINED BACTERIOPHAGE COCKTAIL FOR USE IN HUMAN CLINICAL TRIALS
PLos ONE. 2009;4(3):e4944. Epub 2009 Mar20

Verbeken G., De Vos D., Vaneechoutte M., Merabishvili M., Zizi M., Pirnay J.P.
REGULATORY CONUNDRUM OF PHAGE THERAPY
Future Microbiology 2007 Oct; 2(5): 485-91

Beele H., de la Brassinne M., Lambert J., Suys E., De Cuyper C., Decroix J., Boyden B., Hulstaert F., De Schepper S., Brinssinck J., Delaey B., Draye J-P, De Deene A., Verbeken G.
A PROSPECTIVE MULTICENTER STUDY OF THE EFFICACY AND TOLERABILITY OF CRYOPRESERVED ALLOGENEIC HUMAN KERATINOCYTES TO TREAT VENOUS LEG ULCERS
Extrem Wounds 2005 4:225-233

Duinslaeger LA, Verbeken G, Vanhalle S, Vanderkelen A.
CULTURED ALLOGENEIC KERATINOCYTE SHEETS ACCELERATE HEALING COMPARED TO OP-SITE TREATMENT OF DONOR SITES IN BURNS
J Burn Care Rehabil. 1997 Nov-Dec; 18(6): 545-51

Somers T, Duinslaeger L, Delaey B, Verbeken G, Van Halle S, Boedts D, Govaerts P, Offeciers
STIMULATION OF EPITHELIAL HEALING IN CHRONIC POSTOPERATIVE OTORRHEA USING LYOPHILIZED CULTURED KERATINOCYTE LYSATES
Am J Otol. 1997 Nov; 18(6): 702-6

Somers T, Verbeken G, Vanhalle S, Delaey B, Duinslaeger L, Govaerts P, Offeciers E.
TREATMENT OF CHRONIC POSTOPERATIVE OTORRHEA WITH CULTURED KERATINOCYTE SHEETS
Ann Otol Rhinol Laryngol. 1997 Jan; 106(1): 15-21

De Coninck A, Draye JP, Van Strubarq A, Vanpee E, Kaufman L, Delaey B, Verbeken G, Roseeuw D.
HEALING OF FULL-THICKNESS WOUNDS IN PIGS: EFFECTS OF OCCLUSIVE AND NON-OCCLUSIVE DRESSINGS ASSOCIATED WITH A GEL VEHICLE
J Dermatol Sci. 1996 Dec; 13(3): 202-11

Somers T, Verbeken G, Vanhalle S, Delaey B, Duinslaeger L, Govaerts P, Offeciers E.
LYSATES FROM CULTURED ALLOGENEIC KERATINOCYTES STIMULATE WOUND HEALING AFTER TYMPANOPLASTY
Acta Otolaryngol. 1996 Jul; 116(4): 589-93

Duinslaeger L, Verbeken G, Reper P, Delaey B, Vanhalle S, Vanderkelen A.
LYOPHILIZED KERATINOCYTE CELL LYSATES CONTAIN MULTIPLE MITOGENIC ACTIVITIES AND STIMULATE CLOSURE OF MESHED SKIN AUTOGRAFT-COVERED BURN WOUNDS WITH EFFICIENCY SIMILAR TO THAT OF FRESH ALLOGENEIC KERATINOCYTE CULTURES
Plast Reconstr Surg. 1996 Jul; 98(1): 110-7

Roseeuw DI, de Coninck AL, Vanpée ES, Delaey BM, Verbeken GL, Draye JP.
HEALING OF FULL-THICKNESS WOUNDS TREATED WITH LYOPHILIZED CULTURED KERATINOCYTE CELL LYSATE IN GENETICALLY DIABETIC MICE
Wound Repair Regen. 1995 Oct-Dec;3(4):500-5. Erratum in: Wound Repair Regen. 1996 apr-Jun;4(2):303

10.2.2 Others

Pirnay J.-P., Baudoux E., Cornu O. , Verbeken G. et al
ACCES TO HUMAN TISSUES FOR RESEARCH AND PRODUCT DEVELOPMENT: FROM EU REGULATION TO ALARMING LEGAL DEVELOPMENTS IN BELGIUM
EMBO Reports' Science & Society 2015 May 16(5):557-62. doi: 10.15252/embr.201540070

Co-Author "Chapter in a Book"; Running Title: "Reintroducing Phage Therapy: The Hurdles"; Editors J. Borysowski, R. Miedzybrodzki, A. Górski
REINTRODUCING PHAGE THERAPY IN MODERN MEDICINE: THE REGULATORY AND INTELLECTUAL PROPERTY HURDLES
Caister Academic Press; ISBN 9781908230409, Published April 2014

D. De Vos, M. Merabishvili, G. Verbeken, T. Rose, S. Jennes, J.P. Pirnay
TREATMENT WITH BACTERIOPHAGES: QUID PHAGE THERAPY?
Skin; Vol. 15, N° 4, Sept-Oct. 2012

D. De Vos, G. Verbeken, T. Rose, S. Jennes, J.-P. Pirnay
BACTERIOPHAGES FOR THE TREATMENT OF SEVERE INFECTIONS: A 'NEW' OPTION FOR THE FUTURE?
EWMA Journal 2012; Vol. 12 n° 2

Hilde Stevens, Gilbert Verbeken, Michiel Verlinden, Isabelle Huys
"LOOKING AT CELL- AND GENE THERAPY AS A MEDICINAL PRODUCT": TECHNICAL AND JURIDICAL CHALLENGES DURING ATMP DEVELOPMENT
Tijdschr. Geneeskunde 2011; 67(22):1105-10, 2011, doi:10.2143/TVG.67.22.2001082

Steven Simoens, Gilbert Verbeken, Isabelle Huys
MARKET ACCESS OF BIOSIMILARS - NOT ONLY A COST ISSUE –
Oncologie 2011; 13(5):218-21. doi:10.1007/s10269-011-2018-8

Jean-Paul Pirnay, Alain Vanderkelen, Martin Zizi, Daniel De Vos, Thomas Rose, Geert Laire, Nadine Ectors, Gilbert Verbeken
HUMAN CELLS AND TISSUES: THE NEED FOR A GLOBAL ETHICAL FRAMEWORK
Bull World Health Organ 2010 Nov 1; 88(11):870-2 | PMID: 21076570

Co-Author "Chapter in a Book" / Rapport DKI nr.2009/dk115
"ALTERNATIVES TO ANTIBIOTICS"
Dutch Ministry of Agriculture – Food Quality and Nature / Published 2009

E. Kets, M. Paye, G. Verbeken, P. Coopman, P. Calders, A. Dimitrieff, A. Vanderkelen, D. Roseeuw
TREATMENT OF BURN WOUNDS WITH CULTURED EPIDERMIS
Ann Med Milit Belg. 1991; 5(1):5-10

10.3 Article submitted to international scientific journal

Verbeken G., De Vos D., De Coninck A., Roseeuw D. et al
BACTERIOPHAGE THERAPY: FAST FORWARD TO THE PAST / LESSONS IDENTIFIED FROM THE ADVANCED THERAPY REGULATION
Burns (Submitted 26th March 2015)

10.4 Presentations at conferences and symposia

10.4.1 Oral presentations (Underscore = Presenting Author)

G. Verbeken
CHANGING PHAGE COCKTAILS TO MATCH DEVELOPING RESISTANCE
EMA Workshop on the Therapeutic Use of Bacteriophages, 8th June 2015, Canary Warf, London, UK

G. Verbeken
ADVANCED THERAPY MEDICINAL PRODUCTS IN THE CONTEXT OF HOSPITALS
3th Belgian Symposium on Tissue Engineering (BSTE 2015), Prometheus, 19th March 2015, Arenberg Castel Park, KU Leuven, Belgium

G. Verbeken
EUROPEAN IMPLEMENTATION OF BACTERIOPHAGE THERAPY: IMPACT OF MEDICINAL PRODUCT LEGISLATION ON TAILORED HOPSITAL CARE
Bacteriophage 2015, EuroSciCon, 28 JAN 2015, O_2 - Venue, London, UK

G. Verbeken
BACTERIOPHAGES
Teaching Day, Collegium Chirurgicum Plasticum, 17 JAN 2015, UZ Gent, University Ghent, Belgium

J.-P. Pirnay, C. Ceulemans, D. De Vos, J.-P. Draye, T. Rose, A. Vanderkelen, G. Verbeken
THE COMMERCIALISATION OF HUMAN CELLS AND TISSUES
International Symposium "Globalisation and Commodification of the Human Body: a Cannibal Market?" Foundation Brocher, 6-7 FEB 2014, Geneva, Switzerland

G. Verbeken
HOW QUEENS ASTRIDS' 26-YEARS OLD HUMAN KERATINOCYTE CULTURES BECAME ADVANCED THERAPY MEDICINAL PRODUCTS AT THE DAWN OF THE THIRD MILLENNIUM
Symposium "The Changing World in Cellular Therapies", Free University Amsterdam, 3 Dec. 2013, Amsterdam, The Netherlands & Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

G. Verbeken, G. Verween, B. Pascual, P. De Corte, A. Vanderkelen, T. Rose, S. Jennes, J.-P. Draye, D. De Vos, J.-P. Pirnay1
BANKING SKIN
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

J.-P. Draye, M. Boone, G. Verween, A. Aiti, J.-P. Pirnay, G. Verbeken, D. De Vos, T. Rose, S. Jennes, G. Jemec, V. Del Marmol.
THE USE OF HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY AND REFLECTANCE CONFOCAL MICROSCOPY TO EVALUATE CELLULAR AND ACELLULAR HUMAN DERMAL MATRICES
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

T . Rose, G. Verbeken, S. Jennes, J.-P. Draye, J.-P. Pirnay
THE USE OF HUMAN SKIN PRODUCTS IN BURN WOUND TREATMENT
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

G. Verbeken
HUMAN CULTURED EPITHELIAL ALLOGRAFTS
Cica-démie, 19 Oct. 2013, UC Louvain, Louvain-la-Neuve, Belgium

G. Verbeken, A. Vanderkelen, T. Rose, S. Jennes, D. De Vos, J.-P. Draye, J.-P. Pirnay
HUMAN BODY MATERIAL ™
15th European Burns Association Congress, 28-31 Aug. 2013, Vienna, Austria

J.-P. Draye, M. Boone, G. Verween, A. Aiti, J.-P. Pirnay, G. Verbeken, T. Rose, S. Jennes, G. Jemec, V. del Marmol
ASSESSMENT OF DECELLULARIZED AND RECELLULARIZED HUMAN DERMAL MATRICES USING INVASIVE REAL-TIME HIGH DEFINITION OPTICAL COHERENCE TOMOGRAPHY AND REFLECTANCE CONFOCAL MICROSCOPY
15th European Burns Association Congress, 28-31 Aug. 2013, Vienna, Austria

G. Verbeken
WHAT DOES THE HISTORY OF KERATINOCYTES TEACH US FOR ATMPS?
ATMP-Day, 17 May 2013, KU Leuven, Leuven, Belgium

G. Verbeken
BACTERIOPHAGE THERAPY AND GMP
The Rebirth of Phage Therapy: Why? How? , 31 JAN 2013, Institut Mutualiste Montsouris, Paris, France

G. Verbeken
HOW TO ADAPT THE REQUIREMENTS IN DIRECTIVE 86 TO IMPROVE FEASIBILITY FOR TISSUE BANKS AND STILL GUARANTEE THE SAFETY AND QUALITY OF THE TISSUE PRODUCTS
21st Annual Congress of the EATB, 21-23 NOV 2012, Vienna, Austria

G. Verbeken
DONOR SKIN PROCUREMENT
Annual Congress, SIZ Nursing Association, 13th Oct. 2012, La Marlagne, Wepion, Belgium

G. Verbeken, J.P. Pirnay, R. Lavigne, S. Jennes, D. De Vos, I. Huys
EXPERTS' VIEWS ON THE CURRENT REGULATORY MEDICINAL PRODUCT FRAMEWORK: CALL FOR A DEDICATED EUROPEAN BACTERIOPHAGE THERAPY DIRECTIVE
EuroPhages2012, Bacteriophage in Medicine, Food and Biotechnology, 24-26 SEPT 2012, St Hilda's College, Oxford, UK

G. Verbeken, J.P. Pirnay, S. Jennes, C. Ceulemans, D. De Vos, R. Lavigne, M. Casteels, M. Zizi, I. Huys
BATERIOPHAGE THERAPY: ANALYSES OF SPECIFIC LEGAL HURDLES IN CURRENT REGULATORY FRAMES
Viruses of Microbes (VoM2012), 16-20 July 2012, Royal Military Academy, Brussels, Belgium

I. Huys, G. Verbeken, J.P. Pirnay, D. De Vos, R. Lavigne
INTELLECTUAL PROPERTY RIGHTS IN THE REALM OF PHAGE-BASED PRODUCTS
Viruses of Microbes (VoM2012), 16-20 July 2012, Royal Military Academy, Brussels, Belgium

I. Huys, G. Verbeken, J.P. Pirnay, S. Jennes, D. De Vos., R. Lavigne
PERSPECTIVES IN ANTIMICROBIL AGENTS: PHAGE THERAPY
ESCP International Workshop, 30 May - 1 JUN 2012, Leuven, Belgium

G. Verbeken
CULTIVATION OF NEWBORN FORESKIN KERATINOCYTES: "EPIDERMAL STEM CELLS"?
Cica-démie, 24 March 2012, FUNDP, Namur, Belgium

G. Verbeken
EUROPEAN GOOD TISSUE PRACTICES
Annual Symposium Donation of Organs and Tissues, 8 February 2012, UZ Gasthuisberg, Leuven, Belgium

J. Klykens, J.-P. Pirnay, G. Verbeken, O. Giet, E. Baudoux, R. Jashari, A. Vanderkelen, N. Ectors
CLEAN ROOMS AND TISSUE BANKING: HOW HAPPY I COULD BE WITH EITHER GMP OR GTP
European Association of Tissue Banks, 9-11 November 2011, Barcelona, Spain

G. Verbeken
ALLOGENEIC DONOR SKIN / AN INDISPENSIBLE BIOLOGICAL DRESSING
Organ Donation and Transplantation, 4th Annual Meeting, October 13th 2011, Biopôle, Gosselies, Belgium

G. Verbeken, J.-P. Pirnay, S. Jennes, C. Ceulemans, I. Huys, D. De Vos
BACTERIOPHAGE THERAPY: THE ACTUAL STATUS, A REGULATORY PERSPECTIVE
1st International Oxford Bacteriophage (Phage) Applications Conference, Phages2011, 19-21 September 2011, St Hilda's College, Oxford, U.K.

D. De Vos, M. Merabishvili, G. Verbeken, T. Rose, M. Vaneechoutte, P. Cornelis, R. Lavigne, V. Krylov, A. Dublanchet, I. Huys, S. Jennes, M. Zizi, G. Laire, J.-P. Pirnay
BACTERIOPHAGES AS ANTIMICROBIAL AGAINST MULTIDRUG RESITANT PSEUDOMONAS AERUGINOSA
13th International Conference on Pseudomonas, 4-7 September 2011, Sydney, Australia

H. Stevens, G. Verbeken, M. Verlinden, I. Huys
LEGAL CHALLENGES FOR ATMP DEVELOPMENT
Termis-EU 2011 Annual Meeting, 7-10 June 2011, Granada, Spain

D. De Vos, G. Verbeken, T. Rose, S. Jennes, J.-P. Pirnay
BACTERIOPHAGES FOR THE TREATMENT OF SEVERE INFECTIONS: A 'NEW' WAY FOR THE FUTURE?
EWMA 2011, 25-27 MAY 2011, Brussels, Belgium

G. Verbeken
BACTERIOPHAGE THERAPY: EUROPEAN REGULATORY FRAMEWORK
BACTERIOPHAGE THERAPY: EXPERIMENTS ON HUMANS
GEEPhage, Phagothérapie, Atelier de Travail: « La Phagothérapie en 2011 », 30 March 2011, HIA du Val-de-Grâce, Paris, France

G. Verbeken
DERMAL SUBSITUTES – AN OVERVIEW
Teaching Day Collegium Chirurgicum Plasticum, 26 February 2011, Gent, Belgium

Jean-Paul Pirnay, Gilbert Verbeken
HUMAN CELLS AND TISSUES: THE NEED FOR A GLOBAL ETHICAL FRAMEWORK
Ethical Review in FP7 > "Ethical Issues in Human Cells and Tissues Research", Kick-off Meeting, 14 December 2010, European Commission, Brussels, Belgium

Rob Lavigne, Pieter-Jan Ceyssens, Anneleen Cornelissen, Yves Briers, Maarten Walmagh, Elke Lecoutere, Maia Merabishvili, Gilbert Verbeken, Jean-Paul Pirnay, Daniel De Vos, Mario Vaneechoutte, Stefan Miller, Guido Volckaert
BACTERIOPHAGE-BASED STRATEGIES TO COMBAT PSEUDOMONAS INFECTIONS
Workshop "Current Trends in Biomedicine", 8-10 November 2010, Universidad Internacional de Andalucia, Baeza, Spain

G. Verbeken, D. De Vos, T. Rose, S. Jennes, J.P. Pirnay
SKIN EQUIVALENTS" IN (BURN) WOUND TREATMENT: "REVIEW AND CHALLENGES"
Scientific Meeting, Dutch Society for Burn Wound Care, Friday 5 November 2010, Queen Astrid Military Hospital, Brussels, Belgium

G. Verbeken, D. De Vos, T. Rose, A. Vanderkelen, E. Kets, S. Jennes, J.P. Pirnay
SKIN EQUIVALENTS" IN (BURN) WOUND TREATMENT: "REVIEW AND CHALLENGES"
20th European Tissue Repair Society Congress, 15-17 Sept. 2010, Ghent, Belgium

Daniel De Vos, Maia Merabishvili, Gilbert Verbeken, Thomas Rose, Mario Vaneechoutte, Rob Lavigne, Victor Krylov, Pierre Neirinckx, Serge Jennes, Martin Zizi and Jean-Paul Pirnay
BACTERIOPHAGE THERAPY: THE ROAD TO ACCEPTANCE
"Viruses of Microbes", 21-25 June 2010, Paris, France

D. De Vos, M. Merabishvili, G. Verbeken, T. Rose, M. Vaneechoutte, R. Lavigne, V. Krylov, P. Neirinckx, S. Jennes, M. Zizi, J.-P. Pirnay
BACTERIOPHAGE THERAPY: THE ROAD TO ACCEPTANCE
5th International Conference of the Royal Medical Services, Dead Sea, 3-6 May 2010, Jordan

G. Verbeken, T. Rose, S. Jennes, J. P. Pirnay
NEW EUROPEAN DIRECTIVES ON HUMAN CELL- AND TISSUE BANKING: ETHICAL ASPECTS AND THEIR ECONOMIC IMPACT
European Association of Tissue Banks, 4-6 November 2009, Cracow, Poland,

D. De Vos, T. Rose, S. Jennes, G. Verbeken, J.P. Pirnay
PHAGE THERAPY: FACTS OR FICTION?
European Tissue Repair Society, 2-5 Sept. 2009, Paris, France

G. Verbeken, T. Rose, S. Jennes, J. P. Pirnay
NEW EUROPEAN DIRECTIVES ON HUMAN CELL- AND TISSUE BANKING: ETHICAL ASPECTS AND THEIR ECONOMIC IMPACT
European Burns Association, Lausanne, Switzerland 2-5 September 2009

G. Verbeken, G. Verween, A. De Coninck, T. Rose, D. Roseeuw, S. Jennes, J. P. Pirnay
GLYCEROL TREATMENT AS A BACTERIOLOGICAL DECONTAMINATION PROCEDURE FOR CONTAMINATED ALREADY CRYO PRESERVED DONOR SKIN: METHODOLOGY AND EVALUATION
European Burns Association, Lausanne, Switzerland, 2-5 September 2009

G. Verbeken,
EUROPEAN CLINICAL TRIAL DIRECTIVES / CLINICAL SCIENTIFIC RESEARCH IN BELGIUM / EXPERIMENTS ON HUMANS IN BELGIUM / CLINICAL TRIALS IN BELGIUM
Staff-Training Queen Astrid Military Hospital (QAMH), 24-25-29-30th June 2009, Brussels, Belgium

G. Verbeken, J.P. Pirnay
THE NEW BELGIAN LAW ON CELL- AND TISSUE BANKING: ETHICAL ASPECTS AND THEIR ECONOMIC IMPACT
13th Day of Clinical Biology, Saint-Luc University Hospital (UCL), 9th May 2009, Brussels, Belgium

G. Verbeken[1] , D. De Vos, M. Zizi, M. Vaneechoutte, M. Merabishvili, T. Rose, S. Jennes, J.P. Pirnay
THE CLINICAL USE OF BACTERIOPHAGES: (EUROPEAN) REGULATORY & PSYCHOLOGICAL HURDLES
Phages in Interaction II, Leuven, Belgium, December 19th, 2008

G. Verbeken
SYMPOSIUM "BURN WOUNDS, SKIN DISEASES, CHRONIC WOUNDS AND NOSOCOMIAL INFECTIONS"
Burn Wound Center, Queen Astrid Military Hospital, 25th September 2008, Brussels, Belgium

T. Roelandt, C. Heugheubaert, G. Verween, G. Verbeken, J.-P. Pirnay, D. De Vos, D. Crumrine, D. Roseeuw, P. M. Elias and J.-P. Hachem.
ACTIN / PLASMA MEMBRANE DYNAMICS REGULATE PAR-2-DEPENDENT PERMEABILITY BARRIER RESPONSES TO ACUTE STRESS
International Investigative Dermatology, May 14-17, 2008, Kyoto, Japan

G. Verbeken, M. Vaneechoutte, M. Zizi, T. Rose, S. Jennes, J.P. Pirnay
BACTERIOPHAGES: PHAGE CLINICAL TRIAL VS THERAPY
Phage Biology, Ecology and Therapy Meeting, Eliava 2008, Tbilisi, Georgia

G. Verbeken, T. Rose, S. Jennes, J.P.Pirnay
THE ROLE OF CULTURED HUMAN KERATINOCYTE SHEETS IN THE TREATMENT OF CHRONIC SKIN WOUNDS
Symposium "Chronic Skin Wound Treatment", 2008, Queen Astrid Military Hospital, Brussels

G. Verbeken MSc, P. De Corte BSc, G. Verween BSc, T. Rose MD, S. Jennes MD, J.P. Pirnay
HUMAN CULTURED EPITHELIAL ALLOGRAFTS: INTRODUCING MORE EFFICIENT PRODUCTION SCHEMES AND EXTRA PATIENT SAFETY
TERMIS-EU, June 2008, Porto, Portugal and E.A.T.B., Nov. 2008, Edinburgh, Scotland , U.K.

Thomas Rose, Daniel De Vos, Gilbert Verbeken, Jean-Paul-Pirnay.
NEW BELGIAN GUIDELINES FOR MICROBIOLOGICAL SAFETY SCREENING OF DONOR TISSUE: OPTIMIZED DONOR SKIN SPECIFIC SCREENING PROCEDURE
5th World Congress on Tissue Banking, Kuala Lumpur 2008, Malaysia & E.A.T.B., Nov.2008, Edinburgh, Scotland, U.K.

M. Zizi, G. Verbeken, D. De Vos, M. Merabishvili, N. Chanishvili, T. Rose, S. Jennes, M. Vaneechoutte, J.P. Pirnay
EVALUATION OF A PHAGE COCKTAIL IN THE TREATMENT OF BURN WOUNDS INFECTED WITH MULTI-RESISTANT PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS STRAINS
Symposium "Bacterial Infectiology", Pasteur Institute, 20 Nov. 2007, Paris, France

Verbeken G. , Rose T. , Ortiz S. , Verween G. , De Corte P., Pirson J. , Jennes S.
QUALITY MANAGEMENT SYSTEMS AND TISSUE BANKING
Congress European Burns Association, Budapest, Hungary, Sept. 2007

Verbeken G. , Rose T. , Ortiz S. , Verween G. , De Corte P., Pirson J. , Jennes S.
QUALITY MANAGEMENT SYSTEMS AND TISSUE BANKING "A PRACTICAL APPROACH LEADING TO THE IMPLEMENTATION OF ISO 9001:2000 FOR TISSUE BANKS": UPDATED EXPERIENCE OF THE QUEEN ASTRID MILITARY HOSPITAL
Congress European Association of Tissue banks, Budapest, Hungary, Oct 2007

Verbeken G.
NEW REGULATORY ENVIRONMENT, QUALITY MANAGEMENT SYSTEMS AND TISSUE BANKS: "A PRACTICAL APPROACH"
"Belgian Association of Tissue Banks", Brussels (HCB-KA), April 2007

Ortiz S., Coenye K., Rose T., Verbeken G., De Vos D., Pirnay J.P., Pirson J.
ORGANIZATION OF THE SKIN BANK OF THE BURN WOUND CENTER OF THE QUEEN ASTRID MILITARY HOSPITAL
15th Brussels International Symposium on Vascularised and Non-Vascularised Allografts in Hand/Upper Extremity Surgery, 2007, Brussels, Belgium

Coenye K.E., Verween G., Verbeken G., De Corte P., De Vos D., Vanderkelen A., Pirnay J.P., Pirson J.
CRYOPRESERVED UNDIFFERENTIATED ALLOGENIC KERATINOCYTES AS ADJUVANS TO LARGELY MESHED AUTOLOGOUS SKIN GRAFTS: A FIRST EXPERIENCE
Congress European Association Tissue Banks,Varna, Bulgaria, Oct. 2006

10.4.2 Poster presentations

Verween G., Draye J.-P., A. Aiti, G. Verbeken, D. De Vos, T. Rose, S. Jennes, J-P Pirnay CULTURING AND STORING DIFFERENT HUMAN SKIN CELL TYPES TO REPOPULATE DECELLULARISED HUMAN DERMAL MATRICES
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

Pirnay J.-P., A. Vanderkelen, D. De Vos, J.-P. Draye, T. Rose, G. Verbeken, N. Ectors, I. Huys, S. Jennes, C. Ceulemans
THE EU HUMAN CELL AND TISSUE LEGISLATION SHOULD OVERCOME ETHICAL ISSUES
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

J.-P. Pirnay, A. Vanderkelen, D. De Vos, J.-P. Draye, T. Rose, C. Ceulemans, N. Ectors, I. Huys, S. Jennes, G. Verbeken
BUSINESS ORIENTED EU HUMAN CELL AND TISSUE PRODUCT LEGISLATION WILL ADVERSELY IMPACT MEMBER STATES' HEALTH CARE SYSTEMS
Annual Congress European Association of Tissue Banks, 20-22 NOV 2013, Brussels, Belgium

M. Merabishvili, D. De Vos, G. Verbeken, T. Rose, V. Druez, S. De Roock, P. Soentjens, S. Jennes, J.-P. Pirnay
PHAGE THERAPY RESEARCH AT THE BURN WOUND CENTER OF THE QUEEN ASTRID MILITARY HOSPITAL
15th European Burns Association Congress, 28-31 Aug. 2013, Vienna, Austria

J.-P. Pirnay, A. Vanderkelen, T. Rose, J.-P. Draye, D. De Vos, S. Jennes, G. Verbeken
BUSINESS ORIENTED EU HUMAN CELL AND TISSUE PRODUCT LEGISLATION WILL ADVERSELY IMPACT BURN WOUND TREATMENT

15th European Burns Association Congress, 28-31 Aug. 2013, Vienna, Austria
T. Rose, G. Verween, M. van Brussel, P. Massage, V. Druez, A. Neuprez, E. Keersebilck, G. Verbeken, J.-P. Pirnay, S. Jennes
THE CHALLENGE OF TREATING A 93% TBSA BURNED CHILD
15th European Burns Association Congress, 28-31 Aug. 2013, Vienna, Austria

G. Verween, J.-P. Draye, M.A.L.M. Boone, A. Aiti, G. Verbeken, D. De Vos, T. Rose, S. Jennes, V. del Marmol, G. Jemec, J.-P. Pirnay
CULTIVATION AND STORAGE OF SEVERAL HUMAN SKIN CELL TYPES TO REPOPULATE ACELLULAR HUMAN DERMAL MATRICES
Congress European Tissue Repair Society, 23-25 Oct. 2013, Reims, France

J.-P. Draye, M.A.L.M. Boone, G. Verween, A.-L. Aiti, G. Verbeken, D. De Vos, T. Rose, S. Jennes, J.-P. Pirnay, G. Jemec, V. del Marmol
CELLULAR AND ACELLULAR HUMAN DERMAL MATRICES ASSESSED BY HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY AND REFLECTANCE CONFOCAL MICROSCOPY
Congress European Tissue Repair Society, 23-25 Oct. 2013, Reims, France

Draye J.-P., M. Boone, G. Verween, J.-P Pirnay, G. Verbeken, D. De Vos, T. Rose, S. Jennes, G. Jemec; V. Del Marmol
NONINVASIVE ASSESSMENT OF ACELLULAR DERMAL MATRICES PREPARED BY TWO DIFFERENT METHODS USING NONINVASIVE OPTICAL TECHNOLOGY. HISTOPATHOLOGICAL CORRELATION.
International Investigative Dermatology 2013, May 8th-11th 2013, Edinburgh, Scotland

J.P. Pirnay, G. Verbeken, D. De Vos, J.P. Draye, T. Rose, A. Vanderkelen
BUSINESS ORIENTED EU SAFETY AND HEALTH LEGISLATION UNDERMINES MEMBER STATES' SOCIAL HEALTH SYSTEMS: THE EXAMPLE OF HUMAN CELLS AND TISSUES
21st Annual Congress of the EATB, 21-23 NOV 2012, Vienna, Austria

J.P. Draye, G. Verween, A. vander Straeten, E. Vanderlinden, A. De Coninck, G. Verbeken, D. De Vos, T. Rose, S. Jennes, J.P. Pirnay
PREPARATION OF HUMAN ACELLULAR MATRICES FOR THE DEVELOPMENT OF A LIVING SKIN EQUIVALENT
22nd ETRS Annual Meeting, 4 - 5 OKT 2012, Athens, Greece

G. Verween, J.P. Draye, H. Vroninks, G. Verbeken, T. Rose, S. Jennes, J.P. Pirnay
CULTURING GROWTH RATE IMPROVEMENT OF NEONATAL FORESKIN KERATINOCYTES WITH A NEW DEFINED ANIMAL ORIGIN-FREE CELL CULTURE MEDIUM SUPPELEMENT
22nd ETRS Annual Meeting, 4 - 5 OKT 2012, Athens, Greece

J.P. Pirnay, G. Verbeken, T. Rose, S. Jennes, M. Zizi, I. Huys, R. Lavigne, M. Merabishvili, M. Vaneechoutte, A. Buckling, et al.
INTRODUCING YESTERDAY'S PHAGE THERAPY IN TODAY'S MEDICINE
Viruses of Microbes (VoM2012), 16-20 July 2012, Royal Military Academy, Brussels, Belgium
+ "Bacteriophages and Probiotics – Alternatives to Antibiotics", 1 - 4 JUL 2012, Eliava Institute, Tbilisi, Georgia

M. Merabishvili, D. De Vos, A. Kropinski, R. Lavigne, D. Vandenheuvel, G. Verbeken, M. Vaneechoutte, J.P. Pirnay
CHARACTERIZATION OF NEWLY ISOLATED LYTIC BACTERIIOPHAGES ACTIVE AGAINST ACINETOBACTER BAUMANNII
Viruses of Microbes (VoM2012), 16-20 July 2012, Royal Military Academy, Brussels, Belgium
+ "Bacteriophages and Probiotics – Alternatives to Antibiotics", 1 - 4 JUL 2012, Eliava Institute, Tbilisi, Georgia

M. Merabishvili, P. Wattiau, J. mast, C. ragimbeau, J. Mossong, A. Kropinski, D. Vandenheuvel, R. Lavigne, G. Verbeken, D. De Vos, N. Chanishvili, M. Vaneechoutte, J.P. Pirnay
ISOLATION AND SELECTION OF BACTERIOPHAGES ACTIVE AGAINST ENTEROAGGREGATIVE, SHIGA TOXIN/VEROTOXIN-PRODUCING ESCHERICHIA COLI (EAggEC STEC/VTEC) STRAIN O104:H4
Viruses of Microbes (VoM2012), 16-20 July 2012, Royal Military Academy, Brussels, Belgium
+ "Bacteriophages and Probiotics – Alternatives to Antibiotics", 1 - 4 JUL 2012, Eliava Institute, Tbilisi, Georgia

Verbeken G., Verween G., Pascual B., De Corte P., Rose T., Jennes S., Vanderkelen A., Marichal M., Heuninckx W., De Vos D., Pirnay J.-P.
EVALUATION OF A MICROBIOLOGICAL SCREENING AND ACCEPTANCE PROCEDURE FOR CRYOPRESERVED SKIN ALLOGRAFTS BASED ON 14 DAY CULTURES
European Burns Association, 14-17 September 2011, The Hague, The Netherlands

D. De Vos, M. Merabishvili, G. Verbeken, T. Rose, M. Vaneechoutte, P. Cornelis, R. Lavigne, V. Krylov, A. Dublanchet, I. Huys, S. Jennes, M. Zizi, G. Laire, J.P. Pirnay
BACTERIOPHAGES AS ANTIMICROBIAL AGAINST MULTIDRUG RESISTENANT PSEUDOMONAS AERUGINOSA
13th International Conference on Pseudomonas, 4 – 7 Sept 2011, Sydney, NSW, Australia

Verlinden M., Verbeken G., Huys I.
STUDY OF MODELS FOR 'BIOBANK' PARTNERSHIPS
3rd Annual World Biobanking Summit, 30 June-1 July 2011, Hamburg, Germany

Maia Merabishvili, Gilbert Verbeken, Daniel De Vos, Serge Jennes, Mario Vaneechoutte and Jean-Paul Pirnay
ISOLATION OF BACTERIOPHAGES ACTIVE AGAINST MULTIDRUG-RESISTANT CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII
"Acinetobacter 2010", 1-3 Sept 2010, Rome, Italy

Maia Merabishvili, Jean-Paul Pirnay, Gilbert Verbeken, Daniel De Vos, Nina Chanishvili and Mario Vaneechoutte
STABILITY OF THERAPEUTICALLY APPLICABLE STAPHYLOCOCCUS AUREUS PHAGE ISP IN COMMON ANTIMICROBIALS AND NEUTRAL CARRIERS, "Viruses of Microbes", 21-25 June 2010, Paris, France

Maia Merabishvili, Gilbert Verbeken, Daniel De Vos, Serge Jennes, Mario Vaneechoutte and Jean-Paul Pirnay
ISOLATION OF BACTERIOPHAGES ACTIVE AGAINST MULTIDRUG-RESISTANT CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII
"Viruses of Microbes", 21-25 June 2010, Paris, France (Poster)

G. Verbeken, G. Verween, D. Schoeters, K. Geukens, A. De Coninck, D. Roseeuw, T. Rose, S. Jennes, J. P. Pirnay
POTENTIAL RELEASE OF HEAVY METALS BY FOOD GRADE ALUMINUM FOILS USED FOR SKIN ALLOGRAFT CRYO PRESERVATION
European Burns Association, Lausanne, Switzerland, September 2009
European Association of Tissue Banks, 4-6 Nov 2009, Cracow, Poland

A. Pierlot, S. Ortiz, G. Verbeken, D. De Vos, S. Jennes
DEEP SECOND DEGREE BURNED HAND PALMS OF CHILDREN YOUNGER THAN 2 YEARS NEED CONSERVATIVE SURGERY AND INTENSIVE STRETCHING
European Burns Association, Lausanne, Switzerland, 2-5 September 2009

D. De Vos, T. Rose, S. Jennes, M. Zizi, M. Merabishvili, M. Vaneechoutte, G. Verbeken, J.P. Pirnay
PHAGE THERAPY: FACT OR FICTION?
European Burns Association, Lausanne, Switzerland, 2-5 September 2009

Jean-Paul Pirnay, Thomas Rose, Daniel De Vos, Serge Jennes, Alain Vanderkelen, Nadine Ectors, Gilbert Verbeken
HUMAN DONOR SKIN: NEED FOR EUROPEAN ALLOCATION RULES
European Burns Association, Lausanne, Switzerland, 2-5 September 2009

Maya Merabishvili, Jean-Paul Pirnay, Gilbert Verbeken, Nina Chanishvili, Marina Tediashvili, Nino Lashkhi, Thea Glonti, Victor Krylov, Jan Mast , Luc Van Parijs, Rob Lavigne, Guido Volckaert, Wesly Mattheus, Gunther Verween, Peter de Corte, Thomas Rose, Serge Jennes, Martin Zizi, Mario Vaneechoutte
EVALUATION OF SAFETY ASPECTS OF BACTERIOPHAGE COCKTAIL USED IN THE TREATMENT OF INFECTED BURN WOUNDS IN HUMAN CLINICAL TRIAL
"18th Biennial Evergreen International Phage Biology Meeting", 9-13 August 2009, Washington, U.S.

Pirnay J.-P. , Verbeken G., Rose T., De Vos D., Jennes S., Lismont D., Vanderkelen A. and Ectors N.
HUMAN DONOR SKIN: NEED FOR EUROPEAN ALLOCATION RULES
European Association of Tissue Banks (E.A.T.B.), 12-14 Nov. 2008, Edinburgh, Scotland, U.K.

Pirnay J.-P., Verbeken G., De Vos D., Rose T., Jennes S., Jashari R., Zizi M., Ectors N., Vanderkelen A.
HUMAN CELL AND TISSUE BANKING: NEED FOR A EUROPEAN ETHICAL FRAMEWORK
European Association of Tissue Banks (E.A.T.B.), 12-14 Nov. 2008, Edinburgh, Scotland , U.K.

Merabishvili M., Pirnay J.P., Verbeken G., Chanishvili N., Tediashvili M., Lashkhi N., Krylov V., Mast J., Van Parys L., Lavigne R., Volckaert G., Mattheus W., Verween G., De Corte P., Jennes S., Zizi M., De Vos D., Vaneechoutte M.
QUALITY-CONTROLLED SMALL-SCALE PRODUCTION OF A WELL-DEFINED BACTERIOPHAGE COCKTAIL FOR USE IN HUMAN CLINICAL TRIALS
Phage Biology, Ecology and Therapy Meeting; 12-15 June 2008, Eliava Institute, Tbilisi, Georgia

Pirnay J.P., Merabishvili M., De Vos D., Verbeken G., Coenye K. , Krylov V., Chanishvili N., Zizi M. , Pirson J., Vaneechoutte M.
DEVELOPMENT OF A DEFINED BACTERIOPHAGE COCKTAIL FOR USE IN BURN WOUND PATIENTS
Congress European Burns Association – Budapest – Hungary, 12-15 Sept. 2007
Congress European Association of Tissue Banks – Budapest – Hungary Oct. 2007

De Vos D., Vanderkelen A., Verbeken G., Coenye K., De Corte P., Verween G., Heuninckx W. , Pirnay J.P. , Pirson J.
MICROBIAL SCREENING OF SKIN ALLOGRAFTS: AN UPDATE AND PROPOSITIONS FOR AN OPTIMIZED DONOR SKIN SPECIFIC SCREENING PROCEDURE
Congress European Burns Association – Budapest – Hungary 12-15 Sept. 2007
Congress European Association Of Tissue Banks – Budapest – Hungary Oct. 2007

De Corte P., Verween G., Verbeken G., Pirson J., Pirnay J.P.
FEEDER LAYER- AND ANIMAL COMPONENT FREE CULTURE OF NEONATAL FORESKIN KERATINOCYTES: IMPROVED PERFORMANCE, QUALITY AND SAFETY ASSURANCE
Congress European Burns Association – Budapest – Hungary 12-15 Sept. 2007

De Corte P., Verbeken G., Verween G., Coenye K.E., De Vos D., Vanderkelen A., Pirnay J.P.,
NEONATAL FORESKIN KERATINOCYTES: IMPROVED SAFETY ASSURANCE FOR KERATINOCYTE ALLOGRAFTS
Congress European Association Tissue Banks – Varna- Bulgaria – Oct. 2006

De Vos D., Vanderkelen A., Verbeken G., Coenye K.E., Verween G., De Corte P., Pirnay J.P., Pirson J.
SKIN ALLOGRAFT DISCARDS AS A RESULT OF MICROBIOLOGICAL SCREENING IN THE BRUSSELS SKIN BANK
Congress European Association Tissue Banks – Varna- Bulgaria – Oct. 2006

Verbeken G., De Vos D., Vaneechoutte M., Merabishvili M., Coenye K.E., Pirnay J.P., Pirson J.
BACTERIOPHAGE THERAPY: "TO CLAIM OR NOT TO CLAIM?"
Belgium Society for Microbiology – Brussels – Belgium – Nov. 2006